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ROYAL COMMISSION ON TUBERCULOSIS (HUMAN AND ANIMAL).

SECOND INTERIM REPORT

OF THE

ROYAL COMMISSION

APPOINTED TO INQUIRE INTO THE RELATIONS OF

HUMAN AND ANIMAL TUBERCULOSIS.

PART II. APPENDIX.

VOLUME IV.

COMPARATIVE

HISTOLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS.

BY

ARTHUR EASTWOOD, M.D.

Presented to both Houses of Parliament by Command of His Majesty.

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ROYAL COMMISSION ON TUBERCULOSIS.

COMPARATIVE HISTOLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS

ON

THE RELATIONSHIP OF HUMAN AND BOVINE TUBERCULOSIS.

BY

ARTHUR EASTWOOD, M.D.

[Cd. 3378.]

34727

PREFACE.

This Report contains the evidence provided by my investigations upon the questions with which the Royal Commissioners on Tuberculosis are dealing in their Second Interim Report.

It is divided into three parts. Part I. gives a general account of the nature of my work and of the conclusions at which I have arrived; Part II. contains the details of my researches on the comparative histology of experimental lesions produced by tubercle bacilli from bovine and human sources; and Part III. is a comparative study of the cultural and microscopic characters of bovine and human tubercle bacilli.

From time to time during the progress of the investigations which are recorded in this volume, reports of my work have been submitted to the Royal Commissioners. They have expressed their approval of the methods I have pursued and have left in my hands the detailed organisation of this part of the inquiry. As I am responsible for all the microscopic examinations and comparisons of the morbid processes experimentally produced, and also for the comparison of the cultural characters exhibited by tubercle bacilli of bovine and of human origin, it has been necessary for me to follow closely the course of the animal experiments, both in planning my investigations and in considering my results. The course of the experimental work, as a whole, is dealt with by the Royal Commissioners in their Official Report. It is due to their courtesy that I have been enabled to utilise and discuss the general results of the animal experiments, in so far as they concern my own researches, and so to extend the utility of my histological and bacteriological work by indicating what appears to me to be its value in relation to the experimental inquiry as a whole.

ARTHUR EASTWOOD.

Royalcot Laboratory,
Stansted,
Essex.

August, 1906.

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PART I.

GENERAL REPORT ON THE HISTOLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS.

INTRODUCTION.

The Commissioners have instructed me to make histological examinations and comparisons of the morbid processes set up experimentally by tubercle bacilli of bovine and of human origin; to study and compare the cultural characters of bovine and human tubercle bacilli; and to report to them the results of my observations in so far as they have a bearing on the question of the relationship between bovine and human tuberculosis.

During the last five years this question has been the subject of much controversial discussion. My observations, made without any preconceived theories on the matter, form part of an impartial reinvestigation, *ab initio*, of the whole problem, and are not directly concerned with the merely controversial issues involved. The results at which I have arrived, whilst formulated independently, have also a bearing on these disputed points.

THE HISTOLOGICAL RESULTS.

Subcutaneous Inoculations of Cultures into Calves.

My histological investigations have been made on material obtained from my colleagues, who have conducted the animal experiments. Cultures of bovine bacilli from nineteen different viruses have been inoculated subcutaneously in doses of 50 mg. into one or other of thirty-five calves (including one where the dose was 46 mg.); and cultures from six different bovine viruses have been inoculated subcutaneously in doses of 10 mg. into thirteen calves (including one where the dose was 12·5 mg.). Cultures of human bacilli from fifty-five different viruses have been inoculated subcutaneously in doses of 50 mg. into one or other of 134 calves (including one where the dose was 43 mg. and one where the dose was 45 mg.); and cultures from seventeen of these human viruses, all of which had been found to be of high virulence in doses of 50 mg., were inoculated subcutaneously in doses of 10 mg. into forty-two calves.*

In these experiments, both with 50 mg. and with 10 mg., the animals which had not been fatally infected in less than ninety days were killed as soon as they had survived that period. (There are two exceptions to this statement; one calf inoculated with a bovine virus was killed in apparently good health in fifty-four days, and one calf inoculated with a human virus died in 108 days.)

(1) When inoculated in doses of 50 mg. the viruses of bovine origin which have been tested have all proved fatal in less than ninety days. The same result has been obtained with fourteen viruses of human origin.† There is a

* These statistics refer to experiments which had been completed at the end of August, 1906.

† One calf inoculated with a bovine virus and three inoculated with human viruses did not succumb to the infection; other calves, however, inoculated with the same strains, were fatally infected.

complete histological identity between the lesions produced by these human viruses and the lesions produced by the bovine viruses. The lesions are typical of acute, rapidly progressive tuberculosis.

(2) Besides the above-mentioned fourteen human viruses, certain strains of four additional human viruses* have produced fatal disease in less than ninety days when inoculated in doses of 50 mg. There is evidence that three out of these four viruses exhibit, in the strains referred to, an increase of virulence which is the result of animal passage. It is important to note that the lesions produced by these strains are histologically identical with the lesions produced by the bovine viruses and by those human viruses which were originally of high virulence.

(3) In the fatally infected animals mentioned in (1) and (2) lesions also occur which are representative of the less acute types of the tubercular process; the characteristics of these lesions are that they are circumscribed by fibroblasts, contain few bacilli and generally exhibit many giant cells. The histological unity, therefore, between the progressively destructive and the conservative phases of the tubercular morbid process is established by a comparison of the lesions produced in one and the same animal. Both in calves fatally infected with bovine viruses and in calves similarly infected with human viruses, examples of these less acute lesions are to be found.

(4) In the case of thirty-seven human viruses all the calves inoculated with 50 mg. of culture, in this series of experiments, were killed in apparently good health † after the lapse of ninety days. The same result was obtained in all the experiments made with some of the strains belonging to the four human viruses mentioned in (2). In all these cases it was found either that general dissemination had not occurred, or that the disseminated lesions which were discovered were small, often few in number, and obviously not actively progressive. These lesions are often definitely recognisable, histologically, as tubercles, and tubercle bacilli are sometimes present in them. But, owing to their small size, rarity, retrogressive or not obviously progressive characters, and owing to the scarcity or absence of tubercle bacilli within them, they stand in very marked contrast to the acute and rapidly progressive lesions found in the fatal cases.

(5) Further light is thrown on this very wide difference in the pathogenicity of different viruses, when inoculated subcutaneously in 50 mg. doses of culture, by a comparative study of the processes of infection in their earlier stages. The investigation of calves killed at short periods after inoculation shows that the main issue of the experiment is decided in the subcutaneous tissue at the site of inoculation. This tissue is incapable of offering a successful resistance to a highly virulent virus administered in adequate doses. It is, however, capable of offering against a less virulent virus a resistance which, though not completely successful, is successful to a high degree, and at all events is sufficiently successful to prevent the dissemination of the great proportion of the inoculated material and, consequently, to prevent the reinforcement, from this source, of the bacilli which have been disseminated.

When a virulent virus is inoculated subcutaneously, the tissue at the seat of inoculation offers a vigorous resistance; the bacilli succeed in penetrating the demarcation layer with which the tissue attempts to bar their progress; the resistance on the part of the tissue is continued and again proves unsuccessful; so the process goes on; the resisting tissue which is destroyed from time to time by

* Throughout my Report I have adopted a convenient distinction between the words "strain" and "virus." All bacilli ultimately derived from the same human or bovine subject, viz. material from naturally infected bovines sent from the slaughter-house, or material from infected human subjects sent from the hospitals, are said to belong to the same "virus." Cultures have been raised sometimes from this original material and, in other cases, from experimental animals infected with this material either directly or after previous animal passage. Each of these cultures is regarded as the starting point of a separate "strain." The adoption of this distinction between the two words is particularly useful in discussing cases, such as those mentioned here, where differences have been found between different "strains" originating from the same "virus."

† The few exceptions, where intercurrent disease complicated the experiment, are excluded.

the invading bacilli remains highly vascular until destruction takes place ; consequently frequent opportunities arise for large numbers of bacilli to gain access into the general circulation.

When a less virulent virus is inoculated, the tissue at the seat of inoculation again offers a vigorous resistance ; the bacilli are only to a slight degree successful in penetrating the fibrous tissue barrier which is formed against them ; this barrier soon becomes complete ; the tissue in contact with the bacilli then ceases to be vascularised ; consequently, the opportunities for the bacilli to gain access into the general circulation are relatively small and rapidly diminish. At the site of inoculation the tissue reaction is, within a few weeks, completely successful, and a dense, leathery, fibrous zone is formed which completely debars the entrance of the great mass of the inoculated material into the body. The bulk of the inoculated culture, in fact, is as much external to the body as it would be if it were inclosed within a test-tube.

As a consequence of the different resisting powers of the local lesions, dissemination of bacilli from this source is much more abundant with a virulent than with a less virulent virus, and is maintained for a longer time.

When virulent bacilli are widely disseminated in moderately large numbers, they generally succeed in overcoming the resistance which is offered to them at whatever point in the body they happen to reach ; consequently, they speedily multiply, and acute generalised tuberculosis is the result.

When less virulent bacilli are disseminated in very much smaller numbers, the resistance offered to them at whatever point in the body they happen to reach is generally sufficient either to destroy them, or at least to prevent their rapid multiplication ; consequently, the tubercular foci which are formed are either obliterated or at least do not readily become fresh starting points for the dissemination of the disease.

(6) With regard to the small, disseminated tubercles referred to, histological examination confirms the experimental evidence that the bacilli inoculated were of much lower pathogenicity than the bacilli which have produced fatally progressive lesions. After full emphasis has been laid on this fact, attention may be called to further histological details. About some of these tubercles it may be asserted with confidence that the morbid process has been completely overcome. In some of these cases the tissue damage has been extremely slight and the minute lesion has been overrun with lymphocytes ; there is no fibrous tissue barrier, no caseation, and no calcification. In other instances, again, the lesion is walled in by a very dense, fibrous barrier, enclosed within which there are often found a few calcareous particles. But on the other hand it is not uncommon to find tubercles which, though undoubtedly of the chronic type, cannot be described as "healed." These are centrally caseous and are surrounded by a zone of fibroblasts and other cells. They often contain bacilli. The bacilli, though not very numerous, are sometimes too numerous to justify the assumption that they are merely the relics of bacilli which were actually inoculated and had travelled in the early period of the infection to that particular spot. The original bacilli must have undergone multiplication. Moreover, these caseous foci have certainly expanded since their original formation. There is no proof that, if the animal had been allowed to live, no further expansion would have taken place, nor that the bacilli contained in it would have been annihilated. There is, therefore, the theoretical possibility that such a focus might, later on, have given rise to further dissemination. Taking into account the high resistance of the bovine, when living under favourable nutritive conditions, and the feeble pathogenicity of the bacilli, such a possibility is very remote. At the same time, the fact that these human bacilli of low virulence have survived for ninety days within bovine tissues far remote from the seat of inoculation and are demonstrable within genuine tubercles is evidence of the near relationship between viruses of high and viruses of low virulence for the bovine.

(7) In the exceptional cases where strains, proved by other experiments to be of high or relatively high virulence, have not produced fatal infection in bovines, the lesions found have often been identical with lesions produced by viruses found experimentally to be of uniformly low virulence ; and, when not identical, the lesions have been of a type intermediate in severity between the acute and the chronic.

(8) In the case of the 10 mg. inoculations with bovine viruses, and with human viruses or strains which were known to be highly virulent in 50 mg. doses, fatal infection within ninety days was produced in thirty-nine out of fifty-five experiments. In these fatal cases the lesions are, histologically, of the acute type, closely resembling those produced by 50 mg. of virulent viruses. But, as in the 50 mg. cases, together with these severe lesions, lesions of less severity are found in the same animals; and, in the 10 mg. cases, the occurrence of these lesions of less severity is commoner than in the 50 mg. cases. With regard to the incidence of these variations, there is no difference between the viruses of bovine and those of human origin.

(9) In the remaining sixteen of these 10 mg. experiments the animals survived the ninety days' time limit, and ten of them were reported as apparently "well" when the experiments were terminated. The lesions found in these sixteen animals exhibit very great variety in different cases. This variation ranges from lesions which are nearly as severe as those found in the fatal cases to lesions which are small and more or less retrogressive, resembling lesions produced by the inoculation of 50 mg. of viruses of low virulence. Many instances are provided of the definitely progressve type of circumscribed or "chronic" lesion. It is evident that some of the animals would have died from the disease if the experiment had been continued for a longer period. In the incidence of these variations, there is no difference between the effects of the bovine and the effects of the human viruses inoculated.

(10) The viruses or strains which have produced lesions of the acute, progressive type in these 50 mg. and 10 mg. experiments vary considerably in cultural luxuriance; according to my classification of cultural characters * they fall into one or other of Grades I.–III. The viruses which have consistently failed to produce acute lesions are (with the exception of H 53. D.H., which is classified in Grade III.) of greater cultural luxuriance than the former; they are classed by me in Grades IV.–V.

(11) The general result of this histological investigation is to bring into emphasis two main points ; (1) the marked difference in the severity of the morbid process produced by different viruses ; (2) the underlying histological unity which characterises all these processes, from the mildest to the most severe, as typical of tuberculosis.

Culture Inoculations into Rabbits.

(1) Cultures which are highly virulent for the bovine are also highly virulent for the rabbit, and the histological characters of the lesions produced by them in the rabbit are the same whether the bacilli are of bovine or of human origin.

(2) Those bacilli of human origin which are of low virulence for the bovine are also of relatively low virulence for the rabbit. The only virus which may possibly be regarded as an exception to this rule is H 53. D.H. This virus, derived from a case of lupus, is of high virulence for the rabbit, but of relatively low virulence for the bovine. The lesions produced by it in some of the calves are, however, more extensive than is usual with viruses of low virulence for these animals. Possibly, therefore, the difference in the pathogenicity of this virus for bovines and for rabbits may be explained on the ground that it possesses an intermediate degree of virulence.

(3) The bacilli of low virulence for the bovine are, however, capable of producing tuberculosis in the rabbit. The morbid process set up, though of

* *See* Part III.

much less severity—and, when progressive, of much slower course—is the same in kind as the morbid process set up by more virulent bacilli.

Bovine Lesions Produced under other Experimental Conditions.

In addition to the subcutaneous inoculation of 50 mg. or 10 mg. of culture, other experimental methods have been adopted upon bovines. A large number of animals have been inoculated subcutaneously with bacilli contained in emulsions of animal or human tissue; the bacilli have also been introduced by intravenous injection, by feeding, sometimes by intramammary inoculation, and occasionally by intraperitoneal inoculation. The dose, the duration of the experiment, and the size of the animal have varied in different cases. From the material thus provided I have selected specimens for histological examination. The following are the main results which arise from this part of my work.

The Underlying Unity of the Morbid Process.

The lesions which I have examined, though presenting much diversity in character, are typical of tuberculosis.* They exhibit the features which are admitted by all histologists, whatever their theories as to the histogenesis of the tubercle, to be characteristic of the morbid process known as tuberculosis. There is nothing new or surprising about this fact; it is in harmony with the general consensus of scientific opinion on the histology of this disease. But it is a fact which deserves emphasis, because it has an important bearing on the experimental study of bovine and human viruses. No differentiation of viruses derived from these two sources can be regarded as valid if it fails to take into account the histological unity of the morbid processes which they induce.

The Characteristics of the Bovine Disease.

Under natural conditions bovine tuberculosis is not usually a rapidly fatal disease. In the great majority of cases it runs a very long course; very frequently the disturbance to the general health is no more than slight, and the lesions set up become quiescent or definitely retrogress. This feature of bovine tuberculosis is illustrated by numerous cases of the experimentally produced disease which I have examined. In many instances I have shown that the lesions produced, sometimes after inoculation with a bovine virus and sometimes after inoculation with a human virus, show marked evidence of conservative change; some of these lesions appear to be slowly progressing, but are obviously meeting with a vigorous tissue resistance; others, again, may be described as quiescent; and in many instances the lesions can be definitely pronounced to be retrogressive.

In the second place, the spontaneous disease may assume a highly acute type. To this condition numerous experimental parallels have been obtained, and I have described in detail the histological characters of the lesions produced. The most interesting of these cases are those where the dose inoculated has been kept within moderate limits. These cases show that acute infection may sometimes be set up by introducing into the tissues a number of bacilli which is relatively small, or at least is not enormously greater than the number which is likely to be introduced into the bovine organism by any of the channels of natural infection.

In the third place, infection has been set up in young calves by subcutaneous inoculation with enormous doses of bacilli of varying sources. Under these special experimental conditions the morbid process tends to follow one of two courses. (1) Either the local resistance at the site of inoculation is rapidly overcome, and acute disseminated tuberculosis ensues, or (2) the local resistance is successful in preventing the further invasion of the great mass of the inoculated material, and the relatively few bacilli which become disseminated only succeed in setting up small, chronic and often definitely retrogressive lesions.

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* This statement is not intended to include some of the rapidly fatal results produced by the intravenous inoculation of young calves with large doses.

The Conditions Determining the Severity of Infection.

In comparing my histological results with the experimental conditions under which the lesions I have examined were produced, I find that there are several factors which have helped to determine the degree of severity of the morbid process induced. Taking the subcutaneous inoculations as being the most important, the chief of these conditions are—(1) the natural virulence of the virus ; the differences in the virulence of different viruses have proved to be extremely great, and this is obviously the most important factor : (2) the dose ; *ceteris paribus* a large dose is more likely to produce severe disease than a small dose, but a small dose of a virulent virus may produce severe disease, whereas, with a virus of low virulence, mere increase of the number of bacilli inoculated subcutaneously has not been found to produce disseminated lesions of an acute type : (3) the age of the animal ; it has been conclusively shown that young calves are much more susceptible than older animals : (4) accidental or unknown circumstances, such as "individual susceptibility." To these may be added (5) an indication, based on theory rather than on actual proof, that the condition of vitality of the bacilli inoculated is occasionally a factor of importance ; bacilli which are in a condition of active multiplication when inoculated are more likely to continue to grow quickly than those which are not in this condition, and the more rapidly the bacillus commences its growth the more likely is it to overcome the bovine resistance.*

Progressive and Retrogressive Lesions.

Tuberculosis as a disease is especially characterised by the great variety in the degrees of severity which the morbid process may assume. At the one extreme we have lesions characterised by rapidly progressive necrosis and the presence of large numbers of bacilli ; and at the other extreme we may have lesions which are shown by guinea-pig inoculations to contain no living bacilli ; histologically these lesions may be so far "repaired" as to be no longer definitely recognisable as tubercles, or they may be characterised by a dense fibrous barrier which completely circumscribes a focus of caseous or calcareous material. Between these extremes there are conditions where progress is obviously taking place but more or less slowly, conditions where it is impossible to say whether the lesions have attained their maximum extension or not, and conditions where the conservative changes appear, more or less definitely, to be gaining the upper hand. In other words, just as, clinically, it is often impossible to say definitely whether a case is "cured" or not, so, histologically, it is often impossible to give a categorical "Yes" or "No" in reply to the questions—Has the animal overcome the bacillus or is the bacillus going to overcome the animal ? In summarising my histological results I have endeavoured, as far as possible, to give unambiguous answers to these questions ; but one ought not to forget the common liability of tuberculosis to "recrudesce" from a focus which is apparently quiescent ; and in association with this consideration I call attention to the significant fact that many of these bovine tubercles of the "chronic" or "retrogressive" type not only contain bacilli but are still well vascularised.

Bovine Lesions Produced by Human and by Bovine Viruses.

Amongst the animals inoculated with human viruses I have found every variety of lesion, ranging from the highly acute to the obviously retrogressive ; and amongst the lesions produced by bovine viruses I have found exact counterparts for every one of these. In so far, therefore, as causes which produce identical effects may be held to be identical in character, these investigations provide strong evidence that the bovine bacillus is capable of infecting man.

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* The relatively mild type of disease set up by inoculation of Cow 40 with an old culture of B I. (a virulent virus) may perhaps be taken as an instance in point.

Increase of Virulence due to Residence in Bovine Tissues.

In the case of three human viruses, I have reported on specimens which appear to indicate that their virulence has been increased by repeated residence in the tissues of the bovine.

Susceptibility, Tolerance, and Resistance.

When the animal is highly susceptible to the bacilli inoculated, these multiply freely, and when it is highly resistant they are, as a rule, only found in scanty numbers. There is thus a general relationship, in most cases, between the virulence for a particular animal of the bacilli inoculated and their capacity for multiplication within its tissues. But in certain cases I have found that the bacilli have been able to grow freely, but have produced a relatively small amount of tissue destruction, and the animals have survived the infection for a long period. It is noteworthy that in these cases a very large number of the bacilli are intracellular. This condition may be regarded as illustrating the tolerance of the bovine organism for the bacillus, and may be compared with the same phenomenon which is met with much more commonly in the rat. Cow 74, which was inoculated with a bovine virus, exhibits this tolerance in an interesting manner, and I have also found that in many cases of intravenous inoculation with human viruses of low virulence there is again a very abundant increase of bacilli associated with a relatively small amount of tissue destruction.

Intravenous Inoculations with Human Viruses.

I have examined several specimens of lesions produced in animals, usually calves a few weeks old, which have been inoculated intravenously with large doses of bacilli proved to be of low virulence when inoculated subcutaneously. In some of these cases bacilli are swarming all over the body, and the animal has died in a few weeks, though no lesions have been produced which can be regarded as typical of tuberculosis. In other cases, where the animal has lived longer, bacilli are again very numerous, and lesions have been produced which are more or less characteristic of tuberculosis, though the amount of tissue destruction is usually small relatively to the number of bacilli present. In other cases, again, the animal has successfully resisted the infection, bacilli are scanty, and there is no tubercle formation. And occasionally, as in Calf 361 (H 8. S.C.), typical tuberculosis, histologically of a chronic type, has been produced in all the organs of the body. With regard to the histological interpretation of these results I do not feel prepared to offer a definite opinion. The experimental material provided is not sufficient to determine satisfactorily the susceptibility of the bovine to viruses inoculated by this method. All that can be said about the results obtained is that in some cases death took place too soon to afford any useful indication of the morbid processes at work, in other cases tuberculosis was set up, and in others, again, the animals exhibited a high degree of resistance.

ANTHROPOID APES AND MONKEYS.

These animals have been infected by feeding, by subcutaneous inoculation, and by intravenous inoculation. My histological examinations of the lesions produced support the following conclusions.

Susceptibility.

Anthropoid apes and monkeys are highly susceptible to tuberculosis. This has been shown to be true for every species, including the chimpanzee, upon which an adequate number of experiments has been made. The lesions produced, though varying in severity in different cases, are all typical of tuberculosis.

The Pathogenicity of Different Viruses.

Experiments have been made (1) with bovine viruses which are of high virulence to the bovine and the rabbit; (2) with human viruses, which are of

equally high virulence to these two classes of animals, and (3) with human viruses of much lower virulence. But it has been found, and my histological evidence confirms this result, that anthropoid apes and monkeys are so highly susceptible to the disease that the viruses last mentioned, as well as the two former groups, are capable of producing in these animals severe infection.

There is no evidence that (3) are of greater virulence for either anthropoids or monkeys than (1) or (2). Nor in any other mammals experimentally investigated is there any indication that the viruses with the characters of (3) are of greater virulence than (1) or (2). Therefore the susceptibility of anthropoids and monkeys to (3) cannot be attributed to a selective action of these particular bacilli for this type of animal.

The Modes of Infection.

When the bacilli are introduced by feeding, the results are variable. Dissemination generally occurs and lesions are usually present in the lungs; in fact the incidence of the disease is often particularly severe upon these organs. But the lesions found in these and other distant organs differ very considerably in respect both of their numbers and their histological characters. Sometimes they are scanty and not obviously progressive; at other times they are more numerous and, though of the "chronic" type, exhibit more or less decisive evidence of progression; and at other times again a distinctly acute type of tuberculosis has been produced.

My histological examinations afford evidence that subcutaneous inoculation is a more certain method of producing severe disease, and they also demonstrate the extreme severity of the type of disease which is produced by intravenous inoculation.

Comparison between Anthropoids and Bovines.

My histological work confirms the evidence that the susceptibility of the anthropoid to tuberculosis is intrinsically higher than that of the bovine. Taking the anthropoid as the animal most nearly related to man, the inference is indicated that human susceptibility to this disease is also greater in degree than that of the bovine. This same conclusion is also supported by a consideration of the results obtained in the tests made upon bovines with viruses of bovine and of human origin. All the viruses investigated of bovine origin have been found, when tested under conditions which favour the development of their maximum virulence, to be very highly pathogenic; whereas, of human viruses, tested under equally favourable conditions, some have proved to be of high and others of low virulence. It seems, therefore, when the origins of these viruses are considered, that under the conditions of natural infection, bacilli cannot usually obtain a permanent residence in the bovine unless they are of high virulence; whereas under the conditions of natural infection which prevail for man, bacilli of much lower virulence can also permanently establish themselves and can eventually produce fatal disease.

GOATS.

Severe, progressive disease has been produced in goats both by subcutaneous inoculation and by feeding. The lesions produced by the bovine viruses are identical in histological characters with those produced by the human viruses. All the viruses which have produced lesions of marked severity are known to be of high virulence both to the bovine and the rabbit. Goat 39, which was inoculated from Calf 391, a strain of H 17. Sp.B., did not show acute disease. This strain, as shown by the relatively small amount of disease produced in Calf 391, was of low virulence for bovines. Other viruses or strains of low bovine virulence which have been inoculated into goats have only produced minimal lesions, and no specimens of these have been received for histological examination. Like the bovine, the goat appears to possess a high power of resistance against the bacilli of lower virulence.

Swine.

Disseminated tuberculosis has been readily produced by feeding, and it is noteworthy that the lungs are generally affected. The lesions produced in these feeding experiments are in the majority of cases discrete and of a chronic type, being sometimes apparently retrogressive and sometimes slowly progressive. Less frequently, a much more extensive disease has been produced, and the lesions are characteristic of a severe infection. In all the feeding experiments on which I have reported, bacilli of bovine origin have been administered.

Attention may be called to the characters of the lesions found in two animals which were inoculated subcutaneously. Pig 94 received 1 mg. of a virus (B. IV.) known to be highly virulent to bovines and rabbits, and Pig 23 received 50 mg. of a virus (H 56. F.T.) known to be of much lower virulence for these two classes of animals. In both pigs disseminated tuberculosis was produced, but the small dose of B. IV. produced a distinctly severer type of disease than the large dose of H 56. F.T. But in Pig 23 the tubercles were more numerous than is usually the case in calves which have been inoculated with the same dose of a virus possessing a relatively low degree of virulence for the bovine. On the provisional assumption that the results of these two experiments may be regarded as typical, the pig appears, like bovines, monkeys, and goats, to be highly susceptible to the more virulent bacilli, but to exhibit against bacilli of lower virulence a power of resistance which is rather less than that possessed by the calf, but very much higher than the resistance of anthropoid apes and monkeys.

My histological study of "the process of infection" in pigs shows how rapidly the dissemination of bacilli takes place after subcutaneous inoculation, and may be compared with the investigation which I have reported above on the early dissemination of bacilli in the bovine. These results show that the old theories concerning the resistance offered by the lymphatic glands to the spread of tuberculosis need revision.

Dogs.

When the bacilli are administered by feeding they readily gain access to the lungs, and there set up lesions which are usually not of a very severe type. A much more acute type of disease has been produced by intraperitoneal inoculation, both in Dog 26, which was inoculated with a typical bovine virus, and in Dog 15, which was inoculated with a human virus of low virulence for the bovine.

Most of the feeding experiments have been performed with bovine bacilli of high virulence, but it is noteworthy that in one case feeding with human bacilli (H 25. A.T.), of relatively low virulence for the bovine produced disseminated disease of moderate severity. The dog, therefore, affords another illustration of the identity in kind of the various bovine and human viruses which have been investigated.

In some specimens the bacilli have multiplied very abundantly, but with the production of a relatively small amount of tissue destruction.

Cats.

My histological investigations on the tissues of cats support the experimental fact that infection can be readily produced in the cat both by feeding and by inoculation. The bacilli employed have been, in the cases reported, of bovine origin. In some cases typical tubercles have been produced; in others, the tissue has been swarming with bacilli, but no typical tubercles have been formed.

Rats.

The main feature brought out by my histological examinations is that the rat exhibits a remarkable degree of tolerance towards the tubercle bacillus, both bovine and human. Bacilli may be swarming all over the body, although the amount of tissue damage is extremely slight. Their habit of multiplying within the tissue cells is very noticeable. But in order to propagate the bacillus

within the rat it seems necessary, in most cases, to inoculate very large doses. Moreover, the bacilli do not induce a morbid process which can be histologically described as tuberculosis. The bacillus does not readily kill the rat because the rat is, to a large extent, indifferent to it, not because it evokes the conservative tissue reaction characteristic of the "retrogressive tubercle." The "resistance" of the rat is obviously high, but it is different in kind from the resistance offered by animals such as the bovine and rabbit. All the viruses investigated, including viruses of low virulence for bovines and rabbits, may, under favourable circumstances, multiply freely in the rat. The appearance of tubercle bacilli in the rat is often suggestive of leprosy bacilli.

THE BACTERIOLOGICAL RESULTS.

The Differentiation and Classification of Cultural Characters.

The cultures studied and compared by me have been provided by my colleagues. For the purpose of comparison I have grown them on identical media.

My strains are all kept up on pure serum. For the purpose of differentiating cultural characters I examine the subcultures, made from serum, on broth, glycerin-agar, and potato. Of these last three media I regard glycerin-agar as the most important, and potato as the least; and I think that the three taken together help to control each other, and therefore give more reliable information than any one taken alone.

As a basis of classification, therefore, I take the combined characters which my cultures exhibit on broth, glycerin-agar, and potato.

According to this principle, I have arranged my strains in order, according to their capacity for growth on these media, and commencing the series with those strains which grow least favourably.

I attach high importance to the fact that, when arranged in this way, my strains present one continuous and absolutely unbroken series, passing by gradual and only just perceptible stages of transition from the top of the series, which contains the strains which grow with greatest difficulty, down to the bottom of the series, where are found the strains which grow with greatest luxuriance.

This series I have drawn up in tabular form (pp. 228–232).

As my series makes a very lengthy list, I have divided it up, for the purpose of convenience, into five grades. This subdivision makes it easy to indicate approximately the position in my series of any particular strain, by referring to it as belonging to a certain grade. Within each grade, again, there are differences, the strains at the top growing less well than those at the bottom. My strains have become so numerous that I often find several about which it is impossible to say that one grows better or worse than another. Such strains I group together, arranging them according to the numerical order of the viruses to which they belong. Each group is marked off from the following slightly better growing group by a line drawn across the page.

The bovine and the human viruses are placed in parallel columns, strains with identical cultural characters being opposite each other.

The Comparison of Bovine and Human Strains.

Strains not modified by Animal Passage.

(1) Thirty different bovine viruses have been investigated. In the case of several of these, more than one strain or sample has been examined. The strains have been derived sometimes directly from the original material of the naturally infected bovine which formed the starting point of the virus, sometimes from experimental animals inoculated with this original material, or with cultures of it, and sometimes with experimental animals inoculated with

bacilli which had previously resided in other experimental animals. The total number of strains examined is seventy-seven. Of these strains, five showed definite evidence of modification as the result of experimental animal passage, viz., Baboon 8 (B I.), Dog 72 (B XXII.), Dog 92 (B IV.), Dog 18 (B IV.), and Dog 50 (B IX.). Of the remaining seventy-two, none showed marked evidence of experimental modification.* These seventy-two strains, therefore, may be taken as representative of the natural, unmodified cultural characters of the thirty bovine viruses.

(2) Fifty-seven human viruses have been investigated, and in several instances more than one strain of the same virus has been dealt with. The strains have been derived, sometimes directly from the human material, and sometimes, as with the bovine strains, from experimental animals. The total number of strains examined is ninety-eight. Of these strains, eleven (belonging to the viruses H 13. A.D., H 16. J.H., and H 17. Sp. B.) showed definite evidence of modification as the result of animal passage. The remaining eighty-seven are representative of the natural cultural characters of the bacilli originally resident in the human tissues.*

(3) There are, therefore, available for the purpose of comparison and contrast, seventy-two bovine and eighty-seven human strains.

(4) For each bovine strain an exact parallel in cultural characters is exhibited by one or more of the unmodified human strains, the total number of these parallel human strains being thirty-one.

(5) The thirty-one human strains which are identical with the seventy-two representative bovine strains belong to sixteen different human viruses. With two exceptions, the primary site of human infection in the case of these viruses was regarded as being some portion of the alimentary tract. The two exceptional instances are derived from a case of lupus and a specimen of mixed sputum.

(6) The fifty-six remaining unmodified human strains are different from the seventy-two representative bovine strains. Their difference consists in their possessing a greater degree of cultural luxuriance. With three of these strains, which are placed at the bottom of Grade III., the difference is only slight; with twenty-three, classed in Grade IV., it is greater; and with thirty, classed in Grade V., it is again greater.

(7) The human strains classed in Grades IV.–V. belong to forty-one different viruses. The primary sites of the human infection in these cases are reported as being:—the cervical glands, the mesenteric glands, the bronchial glands, the lungs, the joints, and, in one case, the kidney.

(8) To sum up the above considerations, my first three grades exhibit an almost complete parallelism between the bovine and the human strains; the unmodified strains in Grades IV. and V. are all of human origin.

(9) The unmodified strains which are enumerated in Grades I.–III. provide in themselves, and independently of any reference to or contrast with the strains enumerated in Grades IV.–V., a complete proof that, judged by the criterion of cultural characters, the bovine bacillus is capable of infecting man. The bovine bacilli, in the left hand columns, are representative of the bovine bacillus

* Minor differences frequently occur between different strains of the same virus. For example, different strains of the same virus may be classed in different parts of the same grade, or may occur at the bottom of one grade and at the top of the subsequent grade. These differences have their significance; they illustrate the variability and uncertainty of growth which is characteristic of an organism developing so slowly and so capriciously as the tubercle bacillus, both on culture media and in animal tissues. But it would be misleading to attach much importance to these minor differences as instances of modification.

as usually met with; and the human bacilli, in the right hand columns, which are identical with these, have, in the majority of cases, produced fatal tuberculosis in the human subject. Moreover, this identity of cultural characters is confirmed by identity of experimental virulence.

Strains Modified by Animal Passage.

(1) Five bovine strains, enumerated above, show a decided modification, which is in every case in the direction of increased luxuriance. In two instances this increased luxuriance has brought the strain down into Grade IV., a grade where no unmodified bovine strains occur.

(2) If these modified bovine strains be included amongst the representatives of bovine cultural characters, the number of unmodified human strains with cultural characters parallel to the bovine is increased, and, in addition to cases of alimentary tract infection, includes viruses derived from cases where the primary human infection was reported to be situated in the lungs, bronchial glands, wrist joint, and ankle joint.

(3) Eleven human strains show a marked modification in cultural characters, the modification being in every case in the direction of diminished luxuriance of growth. These strains belong to three viruses. In one of these the human disease was believed to be primary in the bronchial glands, and in another in the knee-joint. The third case was derived from mixed sputum; this last virus was not subjected to modification by animal passage until it had been shown that the bacilli contained in the original material were not heterogeneous in bacteriological characters; they grew with consistently high luxuriance on culture media, and they exhibited, on animal inoculation, consistently low pathogenic properties.

(4) If these modified human strains be regarded from the comparative standpoint, the important fact is established that human viruses, growing with the high degrees of luxuriance exhibited in Grades IV. and V., are capable under favourable animal experiment of acquiring cultural characters identical with those of the unmodified bovine strains classed in Grade I.

(5) To sum up, modification by animal passage breaks down the distinction between Grades I.–III. and IV.–V. as representative respectively of viruses culturally identical with, and different from viruses of known bovine origin.

The Range of Variation in Cultural Characters.

In considering the range of variation in cultural characters it is necessary to fix a standard for each virus. Taking as the standard, representative of each virus, that strain which was isolated directly from the original material, or, when such a strain was not available, the strain which is nearest to the original material, I find that other strains belonging to the same virus sometimes deviate, to a greater or less extent, from the cultural characters of the "standard" strain. These standard or "representative" strains are tabulated, according to their cultural luxuriance, on pp. 234–237; the deviations from them are exhibited in Chart I., at the end of the volume.

It is noteworthy that in all cases where the divergence is very great the modification has been brought about by residence in the tissues of experimental animals. All the cases of great diminution in luxuriance are subsequent to residence in the bovine; the cases of exceptional increase in luxuriance are subsequent, in one instance, to residence in a baboon, in the other instances, to residence in dogs.

The Relation of Cultural Characters to Experimental Virulence for Bovines.

Without entering into finer distinctions and occasional exceptions, it will be sufficient here to deal with a few of the more important facts.

The virulence of the strains in Grades I.–III. is markedly high; that of the strains in Grades IV.–V. is very much lower. In a general way, therefore, there is a parallelism* between cultural characters and virulence, the virulent strains growing less well on artificial media, other than serum, than the slightly virulent. But this parallelism is only roughly true. No gradation in virulence has been found corresponding to the gradation which I have found in cultural characters. For example, many strains in Grade III. have been found as highly virulent as the strains in Grade I.; and many of the strains in Grade IV. have been found as feebly virulent as many of the least virulent in Grade V.

Without entering into the question of intermediate degrees of virulence, and without in any way implying that such intermediate degrees do not exist, it may, then, be stated broadly that whereas there is a gradual and unbroken transition between the cultural characters of the strains which grow least well on artificial media and those which grow most luxuriantly, the transition in the scale of virulence is, on the whole, abrupt. With regard to this difference between culture results and experimental results, some interest attaches to those viruses at the bottom of Grade III. which are of much greater virulence than the viruses at the top of Grade IV., though the difference in the amounts of growth on culture media is only slight. A feature of some significance about the Grade III. cultures is their instability. On broth, for example, the same strain may, on different occasions, produce pellicles of widely different characters. It may form a slightly opaque but thin and uniform pellicle, which refuses to thicken, or a pellicle of moderate but unequal density, or a pellicle which is as uniformly dense as the pellicles produced by Grade V. Another very noticeable character about the broth pellicles formed by Grade III. is that when they grow rapidly and form dense patches they very often become moist and sink. On glycerin-agar and potato there is also some indication of instability amongst these strains. They often begin by growing rapidly and well, as well as Grade IV. strains, but then by about the third week they commence to fall off, as though their original vigour had partially exhausted itself. This lack of cultural stability, therefore, seems to be a feature which distinguishes the better growing virulent strains from less virulent strains.

Apart from Grade III. strains, there are three interesting cases where there is a marked discrepancy between cultural characters and virulence. The strain of H 16. J.H. through Calf 273 was found by me to be in every way typical of Grade V.; but the bacilli had produced disseminated tuberculosis in Calf 273, after subcutaneous inoculation, and the bacilli transferred from the prescapular gland of this animal into the subcutaneous tissue of Calf 355 produced general tuberculosis in the latter animal. There can be no doubt, therefore, as to the virulence of these bacilli for bovines. But the Calf 273 cultures, ten months after isolation, proved only slightly virulent for bovines in doses of 50 mg. This strain, therefore, provides an interesting counterpart to the feature exhibited by the Grade III. strains. Just as highly virulent strains may be unstable in cultural characters, so strains of high cultural luxuriance may be unstable in their degree of pathogenicity. This same characteristic is brought out by the strain of H 13. A.D. through Calf 301. Culturally, I class this strain in Grade IV.; but the bacilli had produced severe generalised tuberculosis in Calf 301, and, when transferred from the tissues of Calf 301 into Calves 321 and 325, produced a similar condition in the latter animals. But cultures of Calf 301 were afterwards found, in 50 mg. doses, to be of distinctly reduced virulence for bovines, whilst cultures of Calf 321, of the same age (fifteen months) and in the same doses, proved very highly virulent. The cultural characters of the Calf 321 strain I have found to be those of Grade I. This example, therefore, illustrates very clearly both instability of virulence and instability of cultural characters. The third interesting example is the strain of H 17. Sp. B. through Rabbit 181. This strain I class at the bottom of Grade IV. in my

* Except in the case of strains which had been long resident on artificial media before I commenced my work, my culture classifications have been made and reported to the Commissioners before the experimental determination of virulence was completed.

culture list. But I find that in the two bovines which were inoculated with 50 mg. of this culture, ten months after isolation, severe generalised tuberculosis was produced. This, then, is another example of the coincidence of marked cultural luxuriance with high pathogenicity.

These observations may be summarised as follows. With the majority of strains there is a general correspondence between cultural characters and virulence, in the sense that those strains which grow poorly on artificial media other than serum are highly virulent, whilst those which grow abundantly on all the artificial media which I have employed are of much lower virulence. But the exceptions to this rule are interesting and important. The relative cultural luxuriance of the Grade III. strains, though most of them are highly virulent, is also an important characteristic. These exceptional cases all exhibit, though in different ways, the characteristic of bacteriological instability. This feature is obviously of high significance in its bearing on the question as to the closeness of relationship between the apparently more stable viruses which diverge from one another in cultural and pathogenic properties.

In order to bring into emphasis the bearing of my results on the general question of the relation of cultural characters to experimental virulence for bovines, I have prepared a series of charts (Charts II.–VIII., see end of volume) which show this relationship in a graphic manner. These charts incorporate the results obtained (1) by the subcutaneous inoculations of 50 mg. of culture into calves, (2) by similar inoculations of 10 mg. of culture, and (3) by subcutaneous inoculations of various doses of tissue emulsions into calves. They also illustrate the modifications observed in the viruses H 13. A.D., H 16. J.H., and H 17. Sp. B.

The Main Facts Illustrated by Charts II.—VIII.

Chart II.: 50 mg. Experiments.—(1) Taking the experiments as a whole, there is a wide difference between the severity of the disease caused by different viruses, the viruses which grow less luxuriantly on culture media being very much more virulent than the viruses which grow more luxuriantly. (2) The latter viruses have in no instance produced a severe type of disease. (3) The viruses which grow with less luxuriance have occasionally, but only rarely, produced a slight amount of disease. (4) Virulence for the bovine, therefore, does not diminish *pari passu* with increase of cultural luxuriance, but shows a sudden drop. (5) Viruses of every degree of cultural luxuriance, from the least to the greatest, have produced true tuberculosis in the bovine, and even with the viruses of least virulence this tuberculosis, though not severe, is often disseminate.

Chart III.: 10 mg. Experiments.—(1) Taking the experiments as a whole, the cultures which are virulent for calves in doses of 50 mg. are also virulent in doses of 10 mg. (2) The virulence curve for 10 mg. falls below the virulence curve for 50 mg. (3) The range of variation in the degree of severity of the disease produced by doses of 10 mg. of bacilli identical in culture characters is much greater than the range with 50 mg. In many instances the animals have survived the 90 days' time limit and have exhibited *post mortem* a less extensive or a relatively slight amount of disease.

Chart IV.: Subcutaneous Inoculations of Tissue Emulsions.—(1) Viruses which produce severe disease when inoculated in the form of 50 mg. of culture are also capable of producing severe disease when inoculated subcutaneously as tissue emulsions. (2) Viruses not capable of producing severe disease (under the period of observation) when inoculated as 50 mg. of culture have not produced severe disease when inoculated subcutaneously as tissue emulsions. (3) The tissue emulsion virulence curve (apparently owing to the smallness of the doses with many of the more virulent viruses) falls below the higher portion of the 50 mg. curve and is also below (though occasionally crossing) the 10 mg. curve. (4) Viruses of every degree of cultural luxuriance, from the least to the greatest, have produced true tuberculosis in the bovine, and even with the viruses of least virulence this tuberculosis, though not severe, is often disseminate.

(5) Viruses of naturally high virulence have in many instances, generally when the dose has been small or when some other circumstance has favoured the animal's resistance, produced disease of no greater severity than that produced by viruses of naturally low virulence for the bovine.

Charts V.–VII. : Experiments Showing Modification.—The experiments made with the viruses H 13. A.D., H 16. J.H., and H 17. Sp. B., show that a complete transformation has been effected from bacilli with high cultural luxuriance and low virulence for the bovine to bacilli with low cultural luxuriance and high virulence for the bovine.

Chart VIII. : The Combined Results of Charts II.–VII.—This chart shows that, taking the sum total of the observations recorded simply as statements of facts, the characteristics of bacilli possessing high virulence for the bovine are interwoven with the characteristics of bacilli possessing low virulence for the bovine, and that no gap is left between them.

Comments on the Above Facts.

(1) These seven charts (Charts II.–VIII.) exhibit in a graphic manner records of facts. These facts may be compared with the theory that mammalian tubercle bacilli are of two distinct and readily separable types, viz., " bovine " bacilli, which are of high bovine virulence and low cultural luxuriance, and " human " bacilli, which possess little or no virulence for the bovine but are of high cultural luxuriance. If the facts recorded had conformed to this theory, Charts II.–VIII. would have been very different. They would have all been alike ; they would have simply shown a short transverse line in the upper part of the left half of each chart and another short transverse line close to the bottom of the right half of each chart.

(2) The facts indicated by these charts could only be reconciled with this theory by adopting the following suppositions. (*a*) The range of variation in cultural characters exhibited by my Grades I.–III. must be disregarded as being of no importance ; the range of variation in cultural characters exhibited by my Grades IV.–V. must be disregarded as being of no importance ; the only fact to be considered of importance about cultures must be the difference between the combined characteristics of Grades I.–III. and the combined characteristics of Grades IV.–V. (*b*) The exceptional instances of the production of only slight disease in calves after subcutaneous inoculation with 50 mg. of viruses belonging to Grades I.–III. must be explained away as due to unusual bovine resistance, or some other factor. (*c*) The numerous instances of the production of relatively slight disease by these same viruses when inoculated in doses of 10 mg. must be dismissed on the ground that 10 mg. is an inadequate dose and cannot be relied on as a test of virulence. (*d*) All the tissue-emulsion inoculations must be discarded except those where viruses belonging to Grades I.–III. have produced severe disease, and those where viruses belonging to Grades IV.–V. have produced no disseminated disease. (*e*) The three viruses which exhibit transformations from one end of the culture and virulence scales to the other must be ignored. (*f*) The fact that the viruses belonging to Grades IV.–V., though of low virulence, produce in the calf a disease which is undoubtedly tuberculosis must be either ignored or in some manner explained away.

(3) It appears to me that such a method of reconciliation would involve a distortion of the facts.

The Nutritive Properties of Various Liquid Media.

The bacteriological results recorded above point to the view that all the viruses dealt with belong to the same family, the differences in cultural characters being merely differences in degree of luxuriance on particular media. I had, therefore, no reason for assuming that an application of the " physiological method," which has been found useful in the differentiation of various nearly allied bacteria, would bring out any marked " physiological " differences between

the various viruses. At the same time I thought it desirable to disregard the indications of my previous work and, on the provisional hypothesis that such physiological differences might exist, to see if I could bring them out.

When an organism grows quickly and vigorously attacks certain carbohydrates or other test substances, with the formation of a well marked and easily recognised change in the reaction of the medium, differentiation from other organisms is easy. But with the tubercle bacillus it is different. Not only does this bacillus grow with great slowness and often with difficulty, but there is no indication, so far as my work goes, that it "attacks" any particular test substance, with the production of readily recognisable decomposition products, in the same way that, for example, many of the micrococci attack and decompose certain of the sugars. It is sometimes assumed that a differentiation of this kind has been found by Theobald Smith, and that his work on glycerin broth cultures shows that the human bacillus forms acid out of that medium, whereas the bovine bacillus does not. If Smith's results * are analysed, it will be found that the final compared with the initial reaction of his medium always showed a diminished acidity and that the advance in the alkaline direction is very variable in amount. If these amounts be tabulated in the order of their numerical values, there is nowhere any gap to be found such as would justify the assumption that we are dealing with a difference in physiological properties, and that the bacilli can be divided into two classes on this basis. The main fact which Smith has established is that when the end reaction shows a marked advance in the alkaline direction the bacillus is probably highly virulent, and when the end reaction shows little or no advance in that direction the bacillus is probably of a low degree of virulence. The total number of human and bovine viruses from which Smith derives his results, in the article to which I refer, is not very large; and an additional difficulty in the way of placing a general interpretation on his conclusions lies in the fact that the weight of the total yield of his broth culture is not stated. Everybody recognises that the average "bovine" bacillus produces on broth a smaller bulk of growth than those human bacilli which are, pathogenically, of less virulence, and culturally, of greater luxuriance. But it is obviously desirable, in order to discriminate between differences in degree and differences in kind, to know whether there is any indication of a direct proportion between final reaction and total yield of growth. It will be noted, in connection with this difficulty, that the "human" bacillus, before it has produced its total yield, moves in its reaction, like the "bovine," in the alkaline direction.

It seems to me, from the experiments I have recorded, that whilst many of the test substances I have used have a marked influence on the growth of the bacilli, and whilst, during the growth of the bacilli, slight variations in the acidity or alkalinity of the medium take place, the formation of free acid or alkali is only a subsidiary event in the life history of the bacillus, and cannot be made the basis of any fundamental distinction.

With regard to the different test substances used. Of the alcohols glycerin stands out conspicuously as being particularly useful in all the media to which it has been added. No encouraging results were obtained with the other alcohols employed. Attention may be called to the marked absence of all beneficial influence with mannite, which in chemical constitution is closely allied to glycerin and to members of the sixth series of sugars, and with dulcite, which is also closely related to the sugars of the sixth series, particularly to galactose. These results may be taken as an indication of the feebleness of the "attacking" power of the tubercle bacillus. Sugars of the sixth series are clearly helpful to the growth of the bacillus, and there seems to be something in the chemical constitution of glucose which renders it rather more adaptable than either galactose or fructose. Compound sugars are very distinctly less useful, another indication of the feeble ability of the tubercle bacillus to split up chemical compounds. As compound sugars are not readily broken up, it is not likely that glucosides would be; in the case of salicin, the phenol element seems to have a distinctly inhibitory effect. The two starches, dextrin and inulin, seem to be to some small extent useful, particularly the former; owing to the complexity of these bodies, it is difficult to find a chemical explanation for this result.

* *Journal of Medical Research*, Feb., 1905, p. 288.

In answer to the question whether these test media throw any additional light on the differentiation of the viruses under examination, I think the reply is that they serve to bring out various points of difference, but that these differences add very little which is new or important to the differences already established by the use of other media. This conclusion, while in the main negative, may also be taken as an indication that the data on which it is based afford some positive evidence in favour of the view that the cultural differences in the viruses examined are merely differences in degree of luxuriance, and do not indicate a more fundamental differentiation into two or more types of bacilli.

The Microscopic Characters of Tubercle Bacilli Obtained from Various Media.

Undiluted Serum.

Bacilli grown on horse, bovine, or dog serum are notably uniform in character. They are usually straight and uniformly stained ; their average length is about 1μ, sometimes rather less and sometimes rather more. Forms at least as short as $\cdot75\mu$ are always present, and frequently there are a relatively small number of organisms which measure from 1·5 to $2\cdot5\mu$. There is no important or constant difference between the strains representative of each of my five grades.

Serum is a good, and with minor exceptions, an equally good, medium for all the strains I have examined. This uniformity of microscopic characters appears to be a consequence of the fact that the bacilli are growing under nutritive conditions which are, approximately, equally favourable to each individual strain. I have recorded in tabular form* the measurements of bacilli, grown on horse serum, which are representative of each of my five grades. The measurements have been made with the aid of a micrometer eyepiece, an Ehrlich's stop, and a mechanical stage. Each bacillus when it comes into the centre of the field is measured, provided that there is no indication that its long axis is not lying at right angles to the line of vision. When the bacilli are dealt with systematically in this way it is found that the longer forms, which more readily attract the eye on a casual inspection, are relatively very few in number.

It will be noted from my table that there is no marked difference between bacilli a fortnight old and bacilli which have been growing for a month.

As a control to this table I have selected, eighteen months after the preparation of the films there recorded, fourteen of the same strains, representative of each of my five grades, and again examined the bacilli taken from fifteen days' horse serum growths. Throughout the eighteen months, the strains had been continuously kept up on serum. In only one specimen was any morphological change noted. This was a film from H 17. Sp. B. (Calf 265). In this case there were many bacilli from 3 to $3\cdot5\mu$ long, and many were curved.

Glycerinated Serum.

Twelve strains from Grade I. have been examined, six from Grade II., six from Grade III., six from Grade IV., and five from Grade V.

The bacilli from Grade I. were straight and uniformly stained ; their length varied from ·75 to $2\cdot5\mu$.

The bacilli from Grade II. were curved and irregularly stained. Their length varied from 1 to $4\cdot5\mu$, exceptional forms being longer.

The bacilli from Grade III. resembled those from Grade II.

The bacilli from Grade IV. were, on the whole, uniformly stained. Curved forms were more numerous than straight. The length of the bacilli varied from 1 to 3μ.

The bacilli from Grade V. resembled those from Grade IV.

Broth.

Out of sixty-one strains from Grade I. forty-nine yielded bacilli which were straight, uniformly stained and varied in length from 1μ, or rather less,

* *See* pp. 257–261.

to about $2 \cdot 5\mu$. In one case no growth was obtained. In the remaining eleven strains the bacilli were longer (1 to 4μ) and both curved forms and irregularly stained forms were frequent.

Twenty-two strains from Grade II. have been examined. In nine of these the majority of the bacilli were curved and irregularly stained; their length varied, in each specimen, from about $1 \cdot 5$ to 4 or $4 \cdot 5\mu$, with occasional longer forms. In the thirteen remaining strains the bacilli were more uniformly stained, shorter (1 to 3 or $3 \cdot 5\mu$), and less frequently curved.

Thirty-five strains from Grade III. have been examined. The characters of the bacilli varied in different strains. In about half, the greater number of bacilli were straight, and in nearly half they were, for the greater part, uniformly stained; when curved the bacilli were generally irregularly stained. The length of the bacilli was also variable. The minimum was rarely under 1μ, but the maximum was in some cases not more than 3μ, and in others between 4 and 5μ.

In Grade IV. (twenty-seven strains) the bacilli generally ranged between 1 and 3 or $3 \cdot 5\mu$ in length, but in five instances they were shorter ($1–2 \cdot 5\mu$), and in two they were longer ($1–5\mu$). In sixteen strains curved forms predominated, and in seventeen strains uniformly stained forms predominated. In the cases where straight forms were in the majority, the bacilli were generally uniformly stained.

In Grade V. thirty strains were examined. The bacilli usually varied in length from 1 to 3 or $3 \cdot 5\mu$. In three cases they were rather shorter and in four rather longer. In twenty-two strains uniformly stained forms predominated, and in eighteen strains the greater number of the bacilli were curved.

Glycerin-Agar.

In Grade I. sixty-one strains were examined. In forty-four of these the bacilli differed only slightly from bacilli grown on serum. They varied in length from $\cdot 75$ to about $2 \cdot 5\mu$, and were on the whole straight and uniformly stained, but curved and irregularly stained forms were somewhat less rare than with serum-grown bacilli. The remaining seventeen strains diverged from the serum type. In eight the divergence was marked; curved and irregularly stained forms were numerous, and the length ranged in some cases from 1 to 3 or 3.5μ, and in others from $1 \cdot 5$ to 5 or 6μ. In the remaining nine, the divergence from the serum type was less marked.

Out of the twenty-two strains in Grade II., ten were curved and irregularly stained and varied in length from 1 to 4μ or occasionally longer; in eight, curved and irregularly stained forms were rather less frequent, and the length was somewhat shorter ($1–3 \cdot 5\mu$); and in four, straight forms predominated, irregular staining was less frequent, and the length varied from 1 to $3 \cdot 5\mu$.

In Grade III. thirty-five strains were examined. In twenty-four the bacilli were curved and irregularly stained; the length was variable; in some cases it ranged from 1, or less, to 3 or 4μ, and in others from 2, or rather less, to 5 or 6μ. In the remaining eleven strains the bacilli were shorter, and straight and uniformly stained forms were much more frequent.

In Grade IV. the bacilli were, on the whole, similar to those in Grade III. Out of twenty-seven strains I noted a predominance of uniformly stained forms in only seven, and a predominance of straight forms in only three. Amongst the irregularly stained forms, regular, discrete beading was more frequent than with Grade III.

In Grade V., out of thirty strains, curved forms predominated throughout, and in only six instances were uniformly stained forms very numerous. The minimum length, in any one film, was sometimes distinctly above 1μ, and, less frequently, rather less than 1μ; the maximum length was never less than 3μ; it was often 4 or $4 \cdot 5\mu$, and occasionally from 6 to 8μ.

Potato.

In Grade I. (sixty-one strains) no growth was obtained in thirteen cases. In thirty-five cases the bacilli resembled serum-grown bacilli, with the exception that slightly longer forms and irregularly stained forms were more frequent. In

thirteen cases the bacilli showed a tendency to become longer, the longest forms measuring from 3·5 to 4μ (rarely 4 to 4·5μ); the staining was less uniform, and curved forms were more frequent.

In Grade II. (twenty-two strains) no growth was obtained in one case. In ten cases the bacilli resembled slightly modified serum-grown bacilli. In the remaining eleven cases the bacilli varied in length from 1 to 4μ (sometimes 5 or 6μ) and were curved and irregularly stained.

In Grade III. (thirty-five strains) the deviation from the serum type was much better marked than in Grade II. In twenty-one cases the bacilli were long, curved, and irregularly stained; in six cases there was a slight divergence from the serum type, and in eight cases there was a somewhat greater divergence from this type.

In Grade IV. curved bacilli predominated in all but three out of twenty-seven strains. Uniformly stained forms predominated in nine strains. The bacilli were notably long. Forms less than 1μ in length were rare, and the minimum length in a film was generally between 1 and 2μ; the maximum length was often from 4·5 to 7μ.

In Grade V. (thirty strains) the bacilli in one case presented very little difference from serum grown bacilli. In twenty-three cases the bacilli were curved and irregularly stained, and varied in length from a minimum of ·75–2μ to a maximum of 3·5–8μ. In the remaining six cases the length was about the same, but curved and irregularly stained forms were rather less frequent. Amongst the irregularly stained forms regular discrete beading was very common.

Summary and Conclusions.

The uniformity in the morphological characters of serum-grown bacilli is notable.

Of bacilli grown on other media the most obvious morphological feature is variety. I do not go so far as to say that my morphological data are merely a hopeless and uninteresting chaos. Some sort of explanation may, I think, be provided which, though it does not amount to a precise scientific system, helps to show that the microscopic appearance of a particular bacillus is not altogether a matter of chance.

I preface this explanation by pointing out that in bacteriology it is generally recognised that morphological features, to use a grandiose term for very minute and relatively structureless organisms, are, in minute respects, highly unstable, and therefore are not regarded as of much diagnostic value. I know of no scientific reason why the tubercle bacillus should be regarded as exempt from the application of this general principle.

I am not referring to the possible occurrence of branched or other peculiar forms, which raise an interesting question of botanical classification. In the films which I have examined branched forms have been very rarely met with, too rarely to attach any special significance to them. It appears, from the work of those who have made a special study of this subject, that various media cause both bovine and human tubercle bacilli to produce branched forms.

In Grade I. the bacilli when transferred from serum to glycerinated serum, broth, glycerin-agar, or potato, show, on the whole, very little microscopic divergence from the serum type. This I regard as evidence of stability. The bacilli grow, though poorly, on these new media, but the media have little power to influence at once their mode of growth.

In Grade II. the bacilli grow on the whole with difficulty, but with less difficulty than Grade I., when transferred to these new media. They are capable of acquiring rather more nutrition out of the media, but this acquisition, being made with difficulty, disturbs them, and the evidence of this disturbance is that they exhibit under the microscope a very much greater divergence from the serum type than Grade I.

In Grade III., where the cultures grow better than in Grade II., but rather capriciously and with lack of stability, there is a still better marked, though somewhat erratic and irregular, divergence from the serum type.

In Grade IV. the bacilli not only diverge from the serum type, they show a tendency to settle down into a different type, the type of bacillus which is much longer than the serum bacillus, shows a well marked curve, and is either uniformly stained or, when stained irregularly, shows a tendency to regular beading.

This tendency to settle down may be associated with the fact that these bacilli grow with greater ease than those of Grade III. on most glycerinated media.

In Grade V. this tendency for the organisms to settle down into the type of bacillus which is long, more frequently curved than straight, and either uniformly stained or regularly beaded, is perhaps a little better marked than in Grade IV.

There is, then, on the whole, an indication that, underlying this great diversity of microscopic features, there is a tendency for the bacillus to express by its morphological condition the nutritive influence of the particular medium upon which it is growing.

The Microscopic Characters of Bacilli in Animal Tissues.

I have exhibited in tabular form * the microscopic characters of an extensive series of bacilli obtained from human and from animal tissues. It will be seen from this table that bacilli from living tissues are longer and less uniform in appearance than bacilli cultivated on serum. It will also be noted that the bacilli which are less highly pathogenic, tend, on the whole, though with notable exceptions, to acquire a rather greater length than the highly virulent bacilli. But though this tendency is recognisable, I think a study of my table will make it sufficiently clear that the virulence of tissue-grown bacilli cannot be diagnosed with any certainty from their morphological appearances.

In addition to the measurements of bacilli recorded in this table, I have paid attention to the microscopic characters of the bacilli present throughout my examinations of histological specimens. These observations support my opinion that morphological appearances cannot be regarded as a reliable guide to diagnosis.

CONCLUSIONS AND SUMMARY.

The general result of my histological work is to emphasise the underlying unity of the morbid processes produced experimentally by infection with every variety of human and bovine tubercle bacillus. These processes show very marked differences in severity, dependent partly upon the dose and the virulence of the bacilli introduced and partly upon the susceptibility of the animals selected for experiment. But even when animals of high resistance, such as calves, are inoculated with bacilli of relatively low virulence, lesions are frequently produced, in situations remote from the site of inoculation, which are typical tubercles and are comparable to the lesions produced in bovines by bacilli of high virulence, when the experimental conditions are such as to favour the resisting powers of the animal. And when the experiments are made on other animals, such as the dog, which also possess a high degree of resistance, though perhaps not so high as that of the bovine, this correspondence of the lesions produced by viruses of different degrees of virulence occurs with much greater frequency. Finally, when highly susceptible animals are used, such as anthropoid apes and monkeys, lesions not only of the more chronic but also of the more acute types appear to be produced with almost equal readiness by these less virulent bacilli.

This underlying unity of the morbid process known as tuberculosis is so frequently taken for granted that its importance is often ignored. Nothing has been done before or since 1901 to invalidate the great mass of evidence which had been accumulated by histologists, both before and after the discovery of the tubercle bacillus, in demonstration of the essential unity, as one and the same disease, of the many phases of the morbid process which tuberculosis may assume. Moreover, the proof that the bacillus of Koch is the cause of tuberculosis is based on the fact that it is constantly found in the lesions histologically known as tubercles.

The differences in virulence between different viruses are brought out in a very striking manner by the subcutaneous inoculation of large doses into young calves. The high degree of resistance which the calf exhibits, in these experiments, towards the bacilli of lower virulence affords of itself a strong presumption that in the processes of natural infection, where much smaller numbers of

* *See* pp. 262–273.

bacilli would be encountered, the bovine tissues would speedily annihilate any of these feebly virulent organisms which might be introduced into the body. This is confirmed by the fact that none of the thirty viruses obtained from spontaneously infected bovines are of low virulence. On the other hand the fact that bacilli of low virulence for the calf have often been obtained from cases of fatal human disease is consistent with the resisting powers of man being less than those of the bovine. This greater degree of susceptibility, on the part of the human subject, has also been shown indirectly by the experiments on the nearest representatives of man, anthropoid apes and monkeys. At the same time it must be recognised that the introduction, irrespective of dose, of virulent bacilli into a susceptible type of animal does not necessarily produce severe disease. This point is illustrated by some of the feeding and a few of the inoculation experiments, conducted upon anthropoid apes and monkeys with bovine bacilli, where a relatively small amount of disease was produced. These cases provide an interesting resemblance between the group of animals nearly related to man and the bovine group. Bovine bacilli, not infrequently when introduced by feeding and sometimes when introduced subcutaneously, may produce in both groups a chronic, latent, or only slowly progressive type of disease.

The method of inoculating 50 mg. of culture subcutaneously into calves is of great diagnostic value in bringing out differences of virulence. The results of this test are mainly determined by the capacity for local resistance which the subcutaneous tissue of the calf possesses. With bacilli of low virulence this resistance succeeds in preventing the great mass of inoculated material from penetrating further into the tissues; whereas with bacilli of high virulence the local resistance fails, and dissemination of the inoculated material is easy. This difference between the success and the failure of the local resistance is further emphasised by the general result, which is, in the one case, survival of the 90 days' time limit, fixed for these experiments, with the production of no more than slight disease, and, in the other, fatal infection in less than 90 days.

But a single diagnostic test, however useful, only provides one out of many items of information which are requisite for the purpose of making an adequate biological study. It is desirable that I should make this point clear. For many experimental purposes this diagnostic test has proved of very high value, and owing to the importance which I attach to it, I place the study and comparison of the lesions produced in bovines by the inoculation of 50 mg. of culture at the commencement of my histological records. But in any serious attempt to study the characteristics of tubercle bacilli isolated from various sources it is obviously necessary to take into consideration all the evidence available concerning the morbid processes induced by the tubercle bacillus when introduced into the animal body (1) by different methods, (2) in different doses, and (3) into different species of animals. It is on the sum total of this evidence that the study of the inter-relationship of human and bovine bacilli must be based, not on the isolated consideration of the effects of 50 mg. inoculations into calves. And on proceeding to the consideration of these other experimental methods, employed both on bovines and on other animals, it at once becomes clear that this particular 50 mg. test, whilst emphasising differences, masks inter-relationship; it fails to bring out the close resemblance which actually exists, both under the conditions of natural infection and under suitable experimental conditions, between the morbid processes set up by strains of bacilli which vary individually in the degree of virulence they possess.

Corresponding with the underlying unity of the morbid processes which these various tubercle bacilli induce, there is a general resemblance in the characters which their growths exhibit on various artificial media. They all grow in the same way, though they differ markedly in the degree of cultural luxuriance which they exhibit; and between the extremes, the viruses which grow very scantily and slowly and the viruses which grow readily and abundantly, every intermediate degree of cultural capacity can be found; just as, histologically, numerous intermediate types of the tuberculous process are demonstrable between the chronic or retrogressive and the acute and rapidly progressive. The bacilli of high pathogenic powers exhibit marked individual differences from each other in their degrees of cultural luxuriance; and, similarly, differences are observable between the viruses of lower virulence; but it is a rule, to which very few exceptions have been found, that viruses of high virulence grow less luxuriantly than viruses of lower virulence.

Growth on artificial media brings out the individuality of a bacillus more strongly, in some respects, than the observation of the morbid processes which it sets up in animal tissues. Taking, for example, the chronic, circumscribed type of tubercle which is commonly met with in bovines, anthropoids, pigs, and dogs, it is impossible to form any opinion as to the cultural characters which the bacilli present in such a tubercle will exhibit; they may occupy any place in the series, from the extreme of scanty growth to the extreme of cultural luxuriance. And, again, with the acute lesions, all we can say is that if the animal in which they have been produced belongs to a naturally resistant species the bacilli will very probably not exhibit a very high degree of cultural luxuriance. But on the other hand, if we take a bacillus which exhibits a particular degree of luxuriance in growth on artificial media, and if this bacillus be passed into animals of many different species, and then recovered from these animals, we find, in the great majority of cases, that the cultural characters of the bacilli so recovered are very much the same as those originally observed. In this respect, therefore, the tubercle bacillus is a remarkably stable organism.

At the same time there is interesting and very important evidence that the cultural and pathogenic characters of at least some of the viruses which I have investigated are not absolutely fixed. Instances have been recorded where there has been (1) a diminution in virulence, (2) an increase of virulence, (3) a diminution in cultural luxuriance, (4) an increase in cultural luxuriance, (5) the association of relatively low cultural luxuriance with relatively low virulence for the bovine, and (6) the association of relatively high cultural luxuriance with high virulence for the bovine and rabbit. These examples, then, illustrate the variability and capacity for modification of the tubercle bacillus.

All these considerations, therefore, tend to show that though there are marked differences in the virulence of different bacilli, in the types of histological lesion produced by identical bacilli, and in the cultural characters of different bacilli, these differences overlap and interweave so closely that it is impossible to find an adequate scientific basis for separating these bacilli into two or more families. These facts indicate that the method of inoculating a very large dose of culture into calves, though very useful for diagnostic purposes, does not, when considered in the light of other established facts concerning the pathogenic and bacteriological characteristics of tubercle bacilli, produce results which justify a separating of these bacilli into two biologically distinct classes of bacteria. With mammalian tubercle bacilli, as with many other bacteria, biologically identical organisms differ widely in pathogenicity.

There seems, then, no valid reason for doubting the opinion, never seriously disputed before 1901, that human and bovine tubercle bacilli belong to the same family. On this view, the answer to the question, Can the bovine bacillus infect man? is obviously in the affirmative. The same answer to this question must also be given by those who adhere to the recent theory that "human" and "bovine" tubercle bacilli are different in kind, since the "bovine kind" are readily to be found as the causal agents of many fatal cases of human tuberculosis.

My work has been especially concerned with the endeavour to ascertain how far it is possible to recognise bacilli isolated from the human body as being of bovine (or other animal) origin. In order to explain how far my results have been helpful, I must point out that there is an important difference between (1) the proof that bovine bacilli are a danger to man; and (2) the identification of such bacilli by bacteriological tests and by microscopic examinations of the morbid processes induced upon experimental animals.

With regard to (1), my work has made the proof conclusive and final. A complete investigation has been made of a large and adequately representative number of bovine viruses, and detailed records have been established of their pathogenicity, the histological characters of the lesions they produce, and the cultural characters of the bacilli. Numerous human viruses have been investigated under strictly identical conditions and have been shown to be identical in every respect with the bovine viruses. The proof, therefore, is based on the simple logical fact that identical causes can produce identical results.

With regard to (2), also, my work supports, to an important extent, the experimental evidence. In the first place, all the thirty bovine viruses which have been investigated appear to be capable of producing severe and fatal disease, with certain definite histological characters, in all animals into the tissues of which they

have been introduced in sufficiently large doses. But there are many human viruses which are not completely identical with these; their main difference lies in the fact that their virulence is markedly less when they are introduced into the subcutaneous tissue of highly resistant animals such as bovines, and the morbid processes induced are found microscopically to be of a very much less severe type. And, together with this lower virulence for the bovine, these bacilli possess a greater degree of cultural luxuriance when grown on artificial media. These two differences, taken in conjunction, afford strong *prima facie* evidence as to whether or not a particular human bacillus is likely to have been derived from an infected bovine (or other animal capable of harbouring similar bacilli). This evidence is particularly valuable owing to the high degree of stability which the tubercle bacillus usually possesses, as shown by the fact that its individual characteristics are not readily changed by transplantation into the tissues of animals of different species. And when it can be shown that the human bacilli which exhibit these two characteristics in common with the bovine gained access to the body by the alimentary tract, the likelihood that they have been derived from a bovine or other animal source is, naturally, still stronger.

Beyond evidence such as I have mentioned I do not think it is possible to go. Apart from differences in the severity of the lesions which the bacilli produce and in the degree of cultural luxuriance which they exhibit, they possess no distinctive features. In microscopic characters, perhaps, the bacilli which grow more abundantly on artificial media tend more frequently to assume curved, uniformly stained, or regularly beaded forms; but these characteristics are so uncertain in their appearance that very little reliance can be placed upon them. It is, in fact, necessary to recognise that we are dealing with bacilli which are so closely related to one another that further differentiation is impossible, for the simple reason that differences, other than those already mentioned, do not exist.

There is another difficulty, pointed out in my study of the relation of cultural characters to virulence, which may sometimes render it impossible to trace with certainty the previous bovine origin of a bacillus isolated from the human body. This difficulty arises from the fact that the tubercle bacillus, though usually an organism which retains its individual characteristics with remarkable tenacity, exhibits at times evidence of instability and a capacity for modification. Experimental and bacteriological evidence of this condition has been provided, and therefore there is good reason to suppose that during the course of long residence in the human body a bacillus, originally of bovine origin, might experience a modification of some of those characteristics which are met with in bacilli freshly isolated from the bovine, and that owing to this modification it might be indistinguishable from bacilli derived from previous cases of human disease. In this connection it is important to remember that a large amount of experimental and histological evidence has been accumulated showing that bacilli introduced by the alimentary tract readily gain access to the lungs.

The general results of my investigations may be briefly summarised as follows:—

1. I have examined and compared the morbid processes induced experimentally by bovine and by human tubercle bacilli, and have found that in animals susceptible to mammalian tuberculosis both the bovine and the human bacilli produce a disease which is typical of tuberculosis.

2. When tested upon the bovine, bacilli of bovine origin produce, in adequate doses, lesions of a highly acute type. When the same tests are applied to bacilli of human origin, some viruses produce in the bovine highly acute lesions identical with those produced by bovine bacilli, whilst other human viruses, under the same experimental conditions, produce only a mild type of disease.

3. The evidence which I have provided with respect to these last mentioned viruses confirms the view that some viruses of human origin are relatively innocuous to bovines.

4. When tested upon anthropoids, which have been selected as being the nearest representatives of man, the bovine bacilli produce, even in small doses, morbid processes which are typical of tuberculosis.

5. Also those human viruses which, in certain tests, have been found relatively innocuous to bovines produce in anthropoids lesions typical of tuberculosis.

6. The disease produced in anthropoids by these latter viruses is not more severe than the disease produced in them by bovine viruses, nor is there any indication that these human viruses have a specially selective action upon anthropoids.

7. Moreover, these human bacilli last mentioned have not been found to produce in any other type of mammal, experimentally investigated, morbid processes which are either more severe than or in any way essentially different from those produced by bovine bacilli.

8. My investigations, therefore, have failed to bring to light the slightest indication that there is any peculiarity about bovine viruses which might suggest their being, compared with certain viruses not of bovine origin, relatively innocuous to the human body.

9. I have examined and compared the cultures, on artificial media, yielded by bovine and by human tubercle bacilli, and find that they all possess in common the characteristics of mammalian tubercle bacilli.

10. The cultures obtained from viruses of bovine origin and the cultures obtained from human viruses have been grown under identical conditions. All the cultures grow in the same way. They differ, however, in the amount of growth produced, the general rule being that those cultures, whether bovine or human, which are of high virulence for bovines grow less abundantly than those human cultures which are of lower virulence for bovines.

11. These differences in the amount of growth produced on certain test media, though not readily changed, are not always stable. In some instances bacilli have exhibited, after residence in the tissues of experimental animals, marked changes in the amount of growth yielded on artificial media.

12. There is, therefore, an essential unity, not only in the nature of the morbid processes induced by human and bovine tubercle bacilli, but also in the bacteriological characters of the tubercle bacilli which cause these processes.

ARTHUR EASTWOOD.

PART II.

THE COMPARATIVE HISTOLOGY OF EXPERIMENTAL LESIONS PRODUCED BY TUBERCLE BACILLI FROM BOVINE AND HUMAN SOURCES.

THE COMPARATIVE HISTOLOGY OF EXPERIMENTAL LESIONS PRODUCED BY TUBERCLE BACILLI FROM BOVINE AND HUMAN SOURCES.

By Arthur Eastwood.

INTRODUCTION.

The histological part of my work has been in progress since January, 1903. I then received instructions from the Commissioners to examine as minutely as possible the microscopic details of the lesions produced by experiments with tubercle bacilli of animal and of human origin, and to institute comparisons between the histological results obtained. This work is not yet completed.

The present Report is concerned with the comparative histology of experimental lesions caused by bovine and by human tubercle bacilli. Its main purpose is to provide the Commissioners with evidence bearing on the questions with which they deal in their Second Interim Report. Having this object in view, I have endeavoured to make my evidence independent of any personal opinion or theory concerning the histogenesis of the tubercle. This is a subject about which the highest authorities on morbid histology disagree, and I think it desirable that I should postpone, until I make my final report, the formulation of my own opinions and theories on these controversial matters. I have therefore endeavoured, in the present Report, to describe my observations in terms which histologists who differ on questions of theory will be able to interpret as expressions of facts. This is not always an easy matter. In morbid processes the normal appearances of cells are often altered, and consequently their nomenclature becomes a matter of difficulty, involving assumptions, which in the case of tuberculosis are frequently matters of dispute, as to the capacity of cells of particular types for undergoing particular modifications. But this difficulty may, with reasonable care, be overcome. By the aid of a few preliminary explanations as to nomenclature, it is possible to describe tubercular lesions in a way which will be intelligible to the rival schools of Baumgarten and Metchnikoff, without involving the acceptance or rejection of any particular theory as to the capacity for transformation of the normal leucocyte, lymphocyte, fibroblast, endothelial cell, or parenchymatous cell. I hope I have met this difficulty in my preliminary explanation of the terminology which I employ.

TECHNIQUE.

The Preparation of Sections.

The pieces of tissue selected, at the post-mortem examination, for histological investigation are placed in 10 per cent. formalin. After being kept in this solution for a few days, the time varying with the size of the pieces, they are well washed in water and transferred to Müller's fluid. This fluid is repeatedly changed, until it remains perfectly clear and ceases to smell of formalin. In order to hasten the process of saturation with Müller's fluid, the bottles are kept for a few days at a temperature of 37°. When it is necessary for me to examine tissues which have already been prepared, by the formalin method, as museum specimens, they are first well washed in water, in order to remove as much as possible of the glycerin, and then treated, as described above, with Müller's fluid. I find that, as a rule, museum specimens after this method of treatment have not lost their capacity for taking up differential stains.

Fixation in formalin, followed by Müller's fluid, is much the best method for general purposes. Formalin is a powerful fixative, which acts quickly and penetrates readily into large pieces of tissue, and though sections fixed with formalin alone do not stain well, the after-treatment with Müller's fluid restores their staining properties. Müller's fluid, on the other hand, though a very useful medium, is much slower in its action and does not fix many of the finer details of cell structure; it cannot, therefore, be advantageously employed in the first stage of the fixing process. Another advantage of the formalin Müller method is that the amount of shrinkage which takes place is relatively small. Still more important is the fact that by this method the red blood-corpuscles are well preserved. It is particularly desirable in dealing with the histology of the tubercle to consider the relation of the morbid process to the vascular supply; but the results obtained after the use of such fixatives as alcohol, which disintegrates the red corpuscles, are apt to be misleading.

I have occasionally made use of other fixatives. The special features of these may be dealt with briefly. Absolute alcohol is a good fixative for the protoplasm of some cells, particularly plasma cells; its disadvantages are that it is necessary to employ thin pieces of tissue, a good deal of shrinkage takes place, and the red blood-corpuscles are disintegrated. Mercuric chloride (a saturated solution in normal saline) is preferable to absolute alcohol; its main disadvantages are the necessity to use very small pieces and the great amount of shrinkage. Müller's fluid saturated with mercuric chloride is a fairly useful fixative, but I do not find that cell details are so well preserved as by the formalin-Müller method. The addition to the former fluid of 5 per cent. acetic acid (Zenker's fluid) has the effect of clearing up the specimens and increasing the sharpness of the staining elements in the nuclei, but I have not found the fixative good for sections which are to be stained for the purpose of differentiating cell details. Flemming's solution is not much use for general purposes. It brings out the fatty deposits in the caseous areas very well. Cell nuclei are fixed well, though no better than with mercuric chloride. The great disadvantage of the fixative is that it damages the properties of the tissue for taking up protoplasmic stains. Neither Pappenheim's stain nor eosin and methylene blue preparations are successful, even after the sections have been bleached with hydrogen peroxide. The Ziehl-Neelsen method of staining preparations fixed in Flemming's solution gives interesting results. Tubercle bacilli stain more darkly than after ordinary fixatives. In many of them definitely black beadings are observed; these beadings appear to correspond in position to the beadings observed in ordinary specimens and not to the more lightly stained material between the beads, as the black spots are frequently terminal as well as intermediate. The black spots do not correspond to "globular swellings"; they are smaller in size, much more abundant, and more uniformly distributed within the bacillus. Carbol fuchsin brings out in almost all the cell nuclei very conspicuous red globules. This point is of some interest with reference to the supposed demonstration by elaborate staining methods of intracellular bodies in certain tissues which have been hardened in some fixative after the type of Flemming's.

When tissues contain only very minute particles of calcareous material decalcification is not necessary. I avoid, as far as possible, specimens containing large masses of calcareous matter, because the requisite decalcifying process interferes with the staining reactions. The decalcifying agent which I like the best is sulphurous acid (saturated watery solution). The tissue, after being fixed in formalin and Müller's fluid, is placed in this reagent and examined from time to time until it is found that the gritty matter has been removed. The process takes several hours if the amount of calcareous deposit is dense. The tissue is then well washed in water and afterwards kept for some days in Müller's fluid, with frequent changes of the liquid.

My specimens are prepared by the paraffin method and are cut with a Cambridge Rocking Microtome (large

pattern). The sections are spread on perfectly grease-free coverslips without the aid of any adhesive.

Staining Methods.

Carbol-Fuchsin and Methylene Blue.—For the purpose of demonstrating bacilli I employ the ordinary Ziehl-Neelsen method. The sections, fixed on coverslips, are stained in the carbol-fuchsin for twenty minutes, with steam gently rising. Bacilli will often take up the stain in a shorter time, or even at room temperature, but I have found that results so obtained are not always reliable; some of the bacilli may be missed. The sections are decolourised with 25 per cent. HCl. I do not consider that this percentage is too high for the employment of the acid-fast test. My experience is that tubercle bacilli will resist for a great many hours the action of this or even a higher percentage of mineral acid. In a few instances I have compared the action of 25 per cent. HCl with that of 1 per cent. acid alcohol. For this purpose I mounted in cedar-wood oil sections which had been decolourised with the acid alcohol, and selected in each a group of bacilli which could be readily identified again; after counting the bacilli, I removed the cedar-wood oil and treated the sections with 25 per cent. HCl. The sections were then remounted and the same groups of bacilli counted again. The numbers on the second count were found to be the same as on the first. There was, therefore, no indication that 25 per cent. HCl decolourised bacilli which were not decolourised by 1 per cent. acid alcohol. In the specimens on which these observations were made it had been found that many of the bacilli present did not take up the carbol-fuchsin stain when exposed to it for a shorter period than twenty minutes.

I think there are important reasons, in applying the acid-fast test, to adhere to the use of a strong mineral acid. It is, of course, a well-known fact that both in tissues and in films from cultures tubercle bacilli are sometimes found which are not acid-fast.

Eosin and Methylene Blue.—For the purpose of demonstrating oxyphil leucocytes I stain for ten to fifteen minutes with 5 per cent. watery eosin and then counterstain with alkaline methylene blue. The method gives good results, but is very troublesome. The main difficulty is to hit off the most suitable strength of methylene blue and the proper time of exposure to its action. These conditions can only be ascertained by actual experiment; they vary with the tissues of different organs and are also affected by the amount of pathological change present in the tissue; for example, in tissue which is engorged with red corpuscles or contains large caseous tracts it is particularly troublesome to remove the excess of eosin without interfering with the protoplasmic stain of the oxyphil leucocytes. This stain is of great service in the detection of oxyphil leucocytes, particularly those of the small, mononuclear type.

Pappenheim's Stain for Plasma Cells.—This is a delicate differential stain of great value. Pappenheim's directions for the use of his stain (*Virch. Arch.* Bd. 166, 1901. S. 424.) are as follows. (1) Stain for two to five minutes in a not very concentrated watery solution of a mixture of methyl green and pyronin. This mixture must be prepared fresh for use every time, according to the following directions; one to two parts, on the point of a penknife, of methyl green and two to four similar portions of pyronin are placed in a test-tube, which is half filled with distilled water. The solution must be of a clear, reddish-violet appearance. (2) Wash for a short time in water. (3) Differentiate in resorcin-alcohol until no more red colour is given off. The resorcin-alcohol solution is prepared by adding to a test-tube a quarter filled with absolute alcohol two to three small portions of resorcin taken up on the end of a spatula. (4) Rapidly dehydrate in absolute alcohol, clear and mount. In my experience this stain, though very troublesome, is extremely useful as a means of differentiating "plasma" cells. For its success it appears to be necessary that the tissue should be fixed when perfectly fresh. In addition to absolute alcohol or mercuric chloride, the two fixatives which are usually recommended, I find that good results can be obtained with formalin-Müller specimens. Before passing the sections into alcoholic resorcin I find it an advantage to leave them for a few minutes in saturated watery resorcin. It is desirable to use distilled water. Mr. Nicholls, my laboratory attendant, who is constantly staining sections for me by this method, often finds it useful to apply gentle heat during the process of staining in the methyl-green-pyronin solution.

Stains for Fibrin.—Weigert's method is notoriously uncertain, and I have not been able to place any reliance on it as a routine method for determining the presence or absence of fibrin in a section. I have adopted the method recommended by Kochel. This is a very much longer process than Weigert's and is not at all easy, but with practice and care it can be made to yield results which are both good and reliable. The method, as used in my laboratory, is the following. (1) Treat the sections for five minutes in 5 per cent. acetic acid; wash. (2) Stain for tubercle bacilli in the ordinary way, but without counterstaining in methylene blue. (3) Place in 5 per cent. chromic acid for about half an hour, until the sections retain a permanent yellow colour after well washing in distilled water. (4) Stain for ten minutes in Weigert's haematoxylin. A longer or a shorter period may be necessary according to the condition of the material and the exact reaction of the stain, which may vary after being made up. The formula for Weigert's haematoxylin is:—haematoxylin, one gram; absolute alcohol, 10 c.c.; distilled water, 90 c.c.; when these ingredients are dissolved add 1 c.c. of a saturated aqueous solution of lithium carbonate. (5) After washing in distilled water, the sections are placed for a short time (about a minute) in a saturated aqueous solution of ammonia alum, until they become a deep blue. (6) After well washing in tap water, the excess of stain is removed by immersion in Weigert's borax-potassium-ferricyanide solution (borax, 2 grams; potassium ferricyanide, 2·5 grams; water 100 c.c.); the time requisite for differentiation varies, according to the tissue, from five minutes or less to about ten minutes. (7) After well washing, the sections are again kept in the alum solution for ten minutes or longer. (8) When the alum has been removed by washing in water, a counterstain, such as thionin, may be used. The sections are then dehydrated and mounted in the usual way.*

The Staining of Elastic and Fibrous Tissue.—For the demonstration of elastic fibres I use the acid orcein method, and for fibrous tissue van Gieson's stain.

TERMINOLOGY.

Leucocytes.—I use the term "leucocytes" in the narrower sense, in which these cells are distinguished from lymphocytes. Leucocytes generally take a protoplasmic stain with eosin in eosin and methylene blue specimens, unless their protoplasm has been damaged and has in consequence lost its staining properties. In describing these cells, reference is frequently made to the shape of the nucleus, the presence or absence of granulation in the protoplasm, and the fineness or coarseness of the granulation, when present.

Small Lymphocytes.—These cells consist of a small, spherical, deeply staining nucleus, surrounded by a very scanty amount of protoplasm. This protoplasm, when demonstrable in eosin and methylene blue specimens, is of a pale blue tinge, never red, and never granular.

Large Lymphocytes.—By these I mean cells with a spherical nucleus surrounded by a large amount of protoplasm which is basophil and not granular.

Plasma Cells.—This name is associated with a controversy which is not yet settled. In order to steer clear of the controversy for the present, and to simplify my descriptions, I define as a "plasma" cell a cell which reacts to a special differential staining reaction. By Pappenheim's staining method, which I follow, the protoplasm takes up a definite red stain, in contradistinction to the protoplasm of all other cells,† which either do not retain the red pyronin stain at all or only show a faint trace of it. In methylene blue specimens the protoplasm of these cells, though not differentiated so well, is characterised by its basophil reaction. The "plasma cell," *i.e.*, the cell which reacts to Pappenheim's differential stain, exhibits very great morphological

* In the employment of this and the two preceding stains I have received valuable assistance from my laboratory attendant, Mr. F. Nicholls. Under my instructions he has learnt all the necessary manipulations, and now stains all my specimens for me.

† Except large lymphocytes.

variety. Sometimes it is indistinguishable from an ordinary large lymphocyte; it is often characterised by the eccentric position of its nucleus; it is often compressed, and rectangular in outline; and it is frequently spindle-shaped. Owing to this diversity in their morphology, it is natural that diverse opinions should be held as to the origin of these cells. Fortunately, the differential staining reaction which they possess in common renders it possible to make a clear distinction between observations of facts and their theoretical interpretation. In the present Report I lay no stress on the theoretical question and do not attempt to solve it, but merely note, from time to time, observations which appear to have a bearing on the problem as to the source from which the plasma cell is derived.

Endothelial Cells.—The possible transformations of the endothelial cell are numerous, and their identification is often a matter of interpretation or personal opinion. When confronted with this difficulty, I have generally introduced the modifying word "apparently." There can, however, be no doubt that the endothelial cell plays an important part in the tubercular process.

Epithelioid Cells.—I frequently use this term, because it has the advantage of bearing a well-recognised morphological significance, without committing one to any particular theory of origin.

SECTION I.

LESIONS PRODUCED UNDER IDENTICAL EXPERIMENTAL CONDITIONS.

CALVES INOCULATED SUBCUTANEOUSLY WITH CULTURE.

I. THE EFFECT OF DOSES OF FIFTY MILLIGRAMMES.

The Commissioners have instituted experiments in each of which a dose of 50 mg.* of a serum culture, estimated volumetrically, has been injected subcutaneously into the neck of a calf. Of these experiments, up to the end of August, 1906, 35 were made with bacilli which were derived from 19 different viruses obtained from naturally infected bovines. In 134 experiments the bacilli were originally derived from human subjects, and represented 55 different human viruses, *i.e.*, were originally obtained from 55 different human beings. A time limit of ninety days was fixed for the experiments, the surviving animals being killed as soon as that period was reached A large number of the calves, however, either died or were killed when moribund before this time limit was reached The experimental results, therefore, may be conveniently divided into two classes, (*a*) where infection proved fatal in less than ninety days, and (*b*) where the animal was killed ninety days after inoculation.

* Occasionally the dose has been rather less than 50 mg.; in these cases, the exact dosage used is stated.

A. INFECTION FATAL IN LESS THAN NINETY DAYS.

Amongst calves inoculated with one or other of the nineteen bovine viruses, fatal disease was produced in 34. Out of these, I have selected for microscopic examination, tissues of the following animals, which are completely representative of the whole series.

Calves Fatally Infected with 50 mg. of a Bovine Virus.

Virus Inoculated.	Number of Calf.	Duration of Experiment in Days.	Virus Inoculated.	Number of Calf.	Duration of Experiment in Days.
I - - - - - -	222	43	VIII. - - - - -	256	37
" - - - - -	230	34	IX. - - - - - -	242	43
II. - - - - - -	212	25	" - - - - -	244	19
" - - - - - -	226	25	X. - - - - -	260	35
III. - - - -	214	45	XI. - - - - - -	232	35
" - - - - -	248	46	" - - - - - -	234	38
IV. - - - - -	218	32	XII. - - - - -	276	24
V. - - -	224	42	XIII. - - - - -	272	46
" - - - - -	246	47	XIV. - - - - -	250	30
VI. - - - -	216	46	" - - - - - -	252	38
" - - - -	330	32	XV. - - - - - -	270	47
VII. - - -	236	34	" - - - - - -	278	41
" - - - - -	238	22	XVI. - - - -	296	32
VIII. - - - - -	254	19	XVII. - - - -	258	18

Calf 218 (B IV.) received a dose of 45 mg., and Calf 216 (B VI.) a dose of 25 mg.

Among calves inoculated with bacilli of human origin, fatal disease was produced in 49. The bacilli were derived from 18 different viruses. As representative of these experiments I have selected tissues from the following animals:—

CALVES FATALLY INFECTED WITH 50 MG. OF A HUMAN VIRUS.

Culture Inoculated.		Number of Calf.	Duration of Experiment in days.
Virus.	Strain.		
2 Sp. A. - - - - - -	G. P. 340 from Heifer 11	569	20
„ - - - - - -	„ „ „	575	33
7 C.M. - - - - - -	G. P. 448 from Calf 5	563	32
„ - - - - -	„ „ „	773	40
10 B.S. - - - - - -	G. P. 757 from Calf 113	387	34
13 A.D. - - - - - -	Calf 321	825	25
14 F.S. - - - - - -	G. P. 587 from O.M.*	545	45
„ - - - - - -	„ „ „	671	33
16 J.H. - - - - - -	Calf 317	711	33
„ - - - - - -	Calf 337	789	49
„ - - - - - -	Calf 423A	541	25
„ - - - - - -	„	559	39
17 Sp. B. - - - - - -	Calf 539	911	58
„ - - - - - -	Calf 553	897	32
„ - - - - - -	Calf 571	739	33
19 S.W. - - - - - -	Heifer 239	577	37
„ - - - -	„	597	45
„ - - - - - -	Goat 11	885	25
20 F.L. - - - -	Calf 213	547	36
„ - - - - - -	„	561	33
28 C.L. - - - - - -	Original Material	489	28
29 M.F. - - - - - -	G. P. 1329 from O.M.*	693	63
„ - - - - - -	„ „ „	697	35
31 L.F. - - - - -	Original Material	519	21
„ - - - - - -	„ „	521	36
32 Y.W. - - - - - -	Human Bronch. Gl.†	837	33
„ - - - - - -	„ „ „	849	23
„ - - - - - -	Human Mesent. Gl.	587	35
„ - - - - - -	„ „ „	589	40
38 J.M. - - - - - -	G. P. 793 from O.M.	643	28
49 T.C. - - - - - -	Original Material	787	81
„ - - - - - -	„ „	957	61
„ - - - - - -	„ „	959	53
„ - - - - - -	Calf 797	931	21
„ - - - - -	„ „	949	39
59 L.B. - - - - - -	Human Lung	965	60
„ - - - - - -	G. P. 1849 from Human Mesent. Gl.	1,063	28
64 M.G. - - - - - -	G. P. 1880 from Human Meninge	1,091	37

* O.M. = Original human material. † Through Rabbit 166.

Calf 521 (H 31. L.F.) received a dose of 30 mg. Calf 1091 (H 64. M.G.) received 45 mg.

GENERAL DESCRIPTION OF MICROSCOPIC DETAILS.

This report deals with the microscopic character of the lesions in the lungs, livers, kidneys, and lymphatic glands of the calves enumerated in the above lists.

In the case of every animal, specimens of each tissue have been examined by special differential staining methods. In their main features, though with minor exceptions subsequently noted, the lesions in the lungs all proved to be of the same type; the lesions in the livers also all conformed to one type; and the same was true for the lesions in the kidneys and in the lymphatic glands. One general description for each organ has therefore been found sufficient to represent the characteristics of the lesions produced in all the animals enumerated above. When any special feature was noticed in any particular animal, or associated with any particular virus, attention has been called to it below, under the title of "Additional Microscopic Details."

LUNGS.

Histological Changes.

General Characters.

All the lungs exhibit a severe type of infection. In all the pieces of tissue examined there are numerous areas of consolidation, and in some of the sections the tissue is almost completely consolidated.

(1) Tubercles, characterised by a central area of commencing or advanced necrosis and a periphery of cells closely packed together and with deeply-staining nuclei, are always present in large numbers. The tubercles are irregular in outline, generally confluent, and show no tendency to demarcate themselves from the surrounding tissue. They often appear to be spreading in all directions by ramifying processes which correspond to the infiltrated and expanded alveolar walls. With rare exceptions no giant cells are found. Not infrequently the necrotic process has extended diffusely in irregular tracts which are too large and too imperfectly separated from the rest of the tissue to be described as definite tubercles.

(2) The alveoli contiguous to the definitely tubercular areas are frequently filled with cellular débris.

(3) Many groups of alveoli are found completely blocked with a non-cellular, coagulated deposit.

(4) The alveolar walls, apart from the tubercular areas, are distended, and the blood capillaries are frequently engorged.

Details.

(1) *Alveolar Epithelium.*—In the tubercular foci most of the alveolar epithelium has disappeared, and in the outlying regions much of it has desquamated and filled the alveolar spaces. There is no evidence that it has proliferated or taken any active part in the tubercular process.

(2) *Oxyphil Leucocytes.*—The oxyphil leucocytes present are all of the multinuclear type. In a few specimens a faint indication of fine granulation can be observed in some of the cells, but with these exceptions the protoplasm is non-granular in eosin and methylene blue specimens.* These cells are present in abnormally large numbers both at the margins of the tubercles and, in the rest of the tissue, amongst the alveolar walls; they also occur in large masses within some of the bronchioles and alveoli. In the areas of commencing necrosis there are also very many nuclear remains probably belonging to cells of this type which have lost their protoplasmic staining properties.

(3) *Small Lymphocytes.*—Cells with small, spherical, deeply-staining nuclei and little or no observable protoplasm are always present in excess of the normal numbers. Apart from the normal nodules of lymphoid tissue, these cells do not occur in very large aggregations. They are generally collected in a loose and irregular manner round the margins of the tubercle and are scattered singly throughout the rest of the tissue. Their numbers vary very considerably in different specimens. On the whole they are abundant, but owing to the uniformity of their distribution do not make a prominent feature in the general structure of the tissue.

(4) *Plasma Cells.*—Cells reaching to Pappenheim's differential stain for plasma cells occur in all the lungs, and in the majority of cases stain well; in the less successful specimens the protoplasmic stain with pyronin is faint, but definitely recognisable.

These cells occur (*a*) amongst the areolar tissue surrounding bronchi, bronchioles, arteries, and veins; (*b*) at the periphery of tubercular areas; (*c*) scattered singly or in small groups amongst the tissue external to the definitely tubercular areas, and more particularly in situations which, as shown by a comparison with sections stained for bacilli, are at the commencement of tubercular changes.

Some of the cells are indistinguishable from ordinary large lymphocytes; some have a circular outline and an eccentric nucleus, and others are compressed, rectangular, or spindle-shaped. The distinguishing feature of all is the differential staining property of their protoplasm.

The following observations have some bearing upon the question as to the origin of these cells:—They are not found in any of their characteristic forms within blood-vessels. They have no definite relationship to the normal lymph nodes. These nodes are generally found situated close to one side of a bronchus, but the plasma cells are found equally distributed all round the bronchus, and there is no evidence of a determination of plasma cells from these lymph nodes to the various situations where plasma cells are found. The plasma cells found in close proximity to bronchi and blood-vessels are very frequently of the compressed type, resembling in outline connective tissue corpuscles with somewhat swollen protoplasm. The theory that plasma cells originate from a transformation of corpuscles normally present in connective tissue is more in accordance with these observations than is the theory that plasma cells are modifications of ordinary lymphocytes circulating in the lymph and blood streams.

(5) *Fibro-blasts.*—There is only a slight tendency for the plasma cells found within or at the margins of the tubercles to assume an elongated form, and the number of other cells, without red protoplasm in Pappenheim specimens, which resemble young connective tissue corpuscles, is generally small. In van Gieson specimens, moreover, there is no evidence of the formation of a fibrous tissue zone round the tubercles. It is obvious that necrotic change is progressing too rapidly to allow of the formation of any permanent fibrous structure.

(6) *Blood-vessels.*—Engorgement of the alveolar blood capillaries is very common, and even in the consolidated areas which have advanced to caseation the vascularity is still considerable. There is no evidence of new capillary formation, such as is frequently found in association with more chronic tubercular lesions.

(7) *Endothelial Proliferation.*—Amongst the heterogeneous mass of cells found in the stage immediately preceding necrotic change there are many which are no doubt of endothelial nature, though it cannot be definitely determined from these specimens that cells of this type have undergone active proliferation.

(8) *Epithelioid Cells.*—Cells which come under this morphological designation are not found, with the exception of desquamated alveolar cells.

(9) *Elastic Fibres.*—Where necrotic change is commencing there is diminution in the amount of elastic tissue; the elastic network is fragmentary, and the individual fibres which survive exhibit some diminution in thickness. These features are well shown in orcein specimens. The erosion of elastic tissue, though unmistakable, has not advanced very far. In the most advanced areas of caseation fragments of this tissue still survive, and in less advanced areas the elastic tissue is almost intact, although the cellular elements have nearly disappeared.

(10) *Fibrin.*—Fibrin, both old and recent, is present in all the specimens, generally in large amount. It is found in the consolidated areas and sometimes in the blood-vessels. In the case of foci where necrotic change has commenced it is more sharply defined at the periphery than in the centre. The distribution of the fibrin is highly irregular. Individual alveoli and groups of alveoli are often found filled with this deposit, whilst the adjacent tissue contains none.

Presence and Characters of Bacilli.

Numbers.

The number of bacilli present differs rather considerably in different cases. Where they are least numerous they do not form dense aggregations, but can always be found without any difficulty in every situation where there is any histological indication of tubercular change, at least a dozen generally appearing in one field under a moderate magnification. In the lungs where they are most numerous they are massed together in sufficiently large numbers to be visible under a low power in most of the areas where necrotic change is commencing. These differences in numbers do not correspond to any differences in the duration of the experiments. The human viruses yield lesions quite as rich in bacilli as the bovine viruses. The lungs infected with H 2. Sp. A., H 38. J.M., H 16. J.H. (Calf 423 A), H 32. Y.W., and H. 20, F.L. are particularly rich in bacilli.

* In more chronic lesions of bovine lungs the granulation of the leucocytes is very marked.

Position.

The bacilli are always found in greatest abundance within, or at the margins of, areas of caseation. Bacilli are found frequently within alveolar epithelial cells, fibroblasts and endothelial cells, and sometimes within multinuclear leucocytes. They are not found within intact blood-vessels. Bacilli are always plentiful in places where there has been a destruction of elastic tissue; but as in these situations there is also advanced tissue change and evidence of broken-down multinuclear leucocytes, it is not clear that the destruction of the elastic fibres is due to the direct action of the bacilli upon them. In specimens stained by carbol fuchsin, followed by orcein and thionin, groups of bacilli are frequently found in close apposition to elastic fibres without any evidence of erosion of the latter. Wherever fibrin is present bacilli are found in association with it, but groups of bacilli are not infrequently found where there is no fibrin.

Characters.

Morphologically, the bacilli, for the most part, vary in length from about 1·5μ to about 3·5μ, the average being from 2·25μ to 2·5μ. Not more than one-fifth exhibit beading or other irregularities of staining. About two-thirds are straight.

There are no appreciable morphological differences between the bacilli derived from different viruses.

LIVERS.

Histological Changes.

General Characters.

The great majority of the livers are characterised by the presence, throughout their substance, of very large numbers of miliary tubercles. The smallest of these are only a few cells in diameter, and the majority of the larger are less than half the area of the microscopic field (low power). Most of tubercles are not sharply marked off from the surrounding liver substance. Occasionally, but not frequently, they contain giant cells. The larger tubercles are centrally caseous. In some cases, but not constantly, there is a small degree of interlobular infiltration.

Details.

(1) *Parenchymatous Cells.*—There is no sign that any of the cells of the liver parenchyma have undergone proliferation. They appear to necrose as soon as a tubercular focus is formed.

(2) *Oxyphil Leucocytes.*—Oxyphil multinuclear leucocytes of the finely granular type are demonstrable in all the livers. They occur within the liver capillaries in numbers which are variable, and are, in many cases, distinctly in excess of the normal. A few can generally be found within or near the margins of the tubercles, and nuclear remains probably derived from these cells are nearly always to be found within the more advanced tubercles. It is doubtful whether these cells play a part of primary importance or specific significance in the formation of the tubercles. When the liver as a whole contains an abundance of circulating leucocytes, these cells are of correspondingly frequent occurrence within the tubercles. When the number of circulating leucocytes is small, the number demonstrable within the tubercles is also small. Comparing tubercles in different stages of development within the same liver as regards their leucocytic content, it is not found that there is any stage which may be characterised as a stage of leucocytic aggregation. If the tubercle is very early and minute, the section of it may or may not pass through one or two leucocytes; if it is larger, some leucocytes, or their nuclear remains, are certain to be demonstrable. In proportion to the area of the foci, the numbers of leucocytes present do not appear to differ very much in these miliary tubercles: the main differences depend on differences in the total number of leucocytes circulating in the liver as a whole. No leucocytes with coarse oxyphil granules are found.

(3) *Small Lymphocytes.*—These cells are almost always to be found in the tubercular areas, but their number is very variable. In some specimens, generally those in which the oxyphil leucocytes are particularly numerous, they are rare; in others they are present in greater numbers than the oxyphil cells. The livers which exhibit some interlobular infiltration contain more small lymphocytes than those which do not. The lymphocytes tend to aggregate round the margins of the tubercles, particularly those which are centrally caseous, and along tracts of interlobular connective tissue; within the non-tubercular liver substance they are scattered singly. In contrast to more chronic lesions, the aggregation of small lymphocytes round or within a tubercle is in no case very dense.

(4.) *Plasma Cells.*—These cells are found both within the tubercular areas and amongst the areolar tissue which surrounds the interlobular vessels. Occasionally, within a blood capillary, a large lymphocyte is to be found which reacts to Pappenheim's stain. The plasma cells are most numerous and conspicuous, and stain most deeply, in the interlobular connective tissue, particularly where this tissue is adjacent to a tubercle. These interstitial plasma cells exhibit every variety of form; many are flattened or elongated, and resemble, in the outlines both of their nucleus and their protoplasm, connective tissue corpuscles. Within the tubercles the plasma cells do not appear able to survive for a long period. They are, however, frequently found even in very early tubercles; and round the margins of those which have advanced to the caseous stage some are almost invariably present. As distinct from the plasma cells found, in these specimens, in contact with the normal connective tissue of the liver, these last-mentioned plasma cells are almost all rounded in outline; though sometimes their protoplasm stains deeply, it more frequently retains the pyronin stain faintly and with difficulty. Mitotic figures are not uncommon. In the case of tubercles which border upon interlobular connective tissue, it is noticeable that the part of their periphery in contact with this tissue is much richer in plasma cells than the rest of the tubercle, and the general appearance strongly suggests that the plasma cells of the tubercle are derived from the connective tissue spaces, but do not survive long after separation from the source which supplies them. There is no evidence of plasma cells demarcating tubercles from the adjacent parenchymatous tissue by the formation of fibroblasts.

(5) *Fibroblasts.*—The tubercles save in exceptional instances, are not bounded by fibrous tissue. In their early stages, before caseation sets in, they contain cells with nuclei like those of young fibroblasts, but these cells soon disappear, and it is difficult to say whether they represent altered capillary endothelial cells or cells of connective tissue origin.

(6) *Blood Capillaries.*—These soon disappear from the tubercles. It is interesting to note that on the rare occasions where giant cells are met with there is also some indication of an attempt at new capillary formation.

(7) *Epithelioid Cells.*—The type of tubercle characterised by the formation of "epithelioid" cells does not occur in these specimens.

(8) *Fibrin.*—Fibrin formation is a very marked feature of these livers. It occurs in almost every specimen examined, forming a deposit which occupies the tubercular foci. In the early tubercles, and at the periphery of the more advanced, the fibrin stains sharply and delicately, being evidently of recent formation.

Presence and Characters of Bacilli.

Numbers.

Baccilli are found without difficulty in all the tubercles, but their numbers are, on the whole, much smaller than in the lungs.

The number of bacilli varies irregularly in different specimens. For example, in the liver of Calf 547 (H 20. F.L.) they are particularly numerous, but this seems to be merely an accident; in the companion animal (Calf 561) they are much less frequent. Again, in the case of the two H 2. Sp. A. calves, the liver of 575 is very much richer in bacilli than the liver of 569. There are also considerable differences amongst the calves inoculated with bovine viruses, the number of bacilli being, on the whole, no greater than after the inoculation of the human viruses.

Position.

As a rule, bacilli are only found within or at the margin of actual tubercles. Occasionally, however, a bacillus is seen within a liver cell; and occasionally bacilli are seen within veins, both portal and hepatic. These intravascular bacilli are sometimes found lying free, and occasionally they are within multinuclear leucocytes, but the majority of them are contained within large cells with vacuolated protoplasm and a large, pale, rounded or somewhat oval nucleus. There is evidence in many places of a desquamation of vascular endothelium, and it is probable that the cells last mentioned are of this type. As regards the bacilli within the tubercles, the cells here are breaking down so rapidly that it is impossible to assert with any confidence how far bacilli are actually included within them; there is no one type of cell with which the bacilli appear to be especially associated.

Characters.

No morphological differences are observable between the bacilli found in the livers and those found in the lungs.

KIDNEYS.

Histological Changes.

General Characters.

The kidneys are much less extensively affected than the livers. Whereas in the livers microscopic tubercles are scattered at short intervals throughout the sections examined, in the kidneys the tubercular foci are comparatively few; often only one or two are found within a section, and rarely more than half a dozen.

(1) The larger tubercles appear to the naked eye as small grey points, generally situated in the cortex. Under the microscope these tubercles are centrally caseous, and are sharply marked off from the rest of the tissue by a dense zone of cells, the larger number of which have round, deeply-staining nuclei.

(2) More frequently than the above lesions there are found minute areas of commencing caseation in the midst of small tracts of interstitial infiltration. These lesions are not visible to the naked eye.

(3) Small patches of interstitial infiltration occur without any caseation or other histological evidence that the lesion is tubercular.

(4) There are no giant cells.

Details.

(1) *Renal Epithelium.*—There is no evidence that the renal epithelium proliferates or takes any active part in the tubercular process.

(2) *Oxyphil Leucocytes.*—Finely granular multinuclear leucocytes can be demonstrated in almost all the lesions, but, with few exceptions, only in very small numbers. In the exceptional cases the number of these cells present in the tubercles, though greater, is not very large; and a slight general leucocytosis can be observed throughout the renal tissue.

(3) *Small Lymphocytes.*—These cells occur in all the tubercles and in the areas of infiltration; they are much more numerous than the oxyphil leucocytes.

(4) *Plasma Cells.*—Plasma cells are invariably present in association with the tubercular areas. The centrally caseous miliary tubercles are always surrounded with a dense zone of these cells, and plasma cells are also abundant in the interstitial infiltration which shows evidence of commencing tubercular change. In the rest of the interstitial tissue, where there is no histological sign of tubercular infection, plasma cells are sometimes met with, either singly or in short chains; but the occurrence of these isolated plasma cells is exceptional. Where no tubercles are present, and where there is no increase of interstitial tissue, plasma cells are, as a rule, absent. Surrounding the tubercles, the plasma cells are generally rounded or somewhat oval in outline; in the interstitial tissue compressed or elongated forms are more frequent and the plasma cells which occur apart from obvious lesions are not infrequently spindle-shaped, like ordinary connective tissue corpuscles, except for the red colour of their protoplasm. Very occasionally a cell, apparently a large lymphocyte, which reacts to Pappenheim's stain, is found within a glomerulus.

(5) *Fibroblasts.*—Although the boundary of the tubercles consists of a dense cellular layer, very little fibrous tissue is found in this situation. The boundary zone consists mainly of plasma cells and small lymphocytes which can be traced outwards for a short distance into the renal interstitial tissue. Within the tubercles the tendency is to necrosis, and not to the formation of fibrous tissue. And in general, where the renal interstitial tissue is increased, the increase is due largely to the presence of plasma cells and small lymphocytes, and only slightly to the reproduction of normal connective tissue.

(6) *Glomeruli.*—All the kidneys examined support the view that in the bovine kidney the tubercular process begins not in the glomerulus but in the interstitial tissue. As the process extends the renal tubules and the glomeruli become involved and disappear, the latter tending to survive longer than the former. The earliest lesions are never found within a glomerulus.

(7) *Blood-vessels.*—The capillaries tend to become obliterated in the areas where the tubercular process is advanced. There is no evidence of the formation of new vessels.

(8) *Endothelial Cells.*—There is no evidence of a proliferation of endothelial cells, nor is there a formation of any cells of " epithelioid " type.

(9) *Fibrin.*—A trace of fibrin is sometimes found, but only rarely. The absence of fibrin in most of the lesions is in marked contrast to the condition of the livers.

Presence and Characters of Bacilli.

Numbers.

Tubercle bacilli are demonstrable in most of the lesions, but in some cases are rare. On the whole, they are less numerous than in the livers. Occasionally, however, caseating tubercles are found which contain bacilli in such large numbers that they are visible with a low power. The general tendency is for the tubercles as they increase in size to contain more bacilli. This is an important difference from tubercles in chronic infections, and should be associated with the fact that the cellular zone which surrounds these tubercles, though well defined, contains very little fibrous tissue. The kidneys of Calf 575 (H 2. Sp. A.) and Calf 643 (H38. J. M.) show particularly large numbers of bacilli; but in some of the kidneys infected with bovine viruses (Calf 214 and Calf 224), where the sections happen to pass through fairly large tubercles, the bacilli are also numerous, and there is no reason to suppose that there is any difference between the viruses as regards capacity of the bacilli to multiply in the kidney.

Position.

Bacilli are constantly found within the miliary tubercles and at the margin of these tubercles. A few are generally found in the areas of interstitial infiltration which show a tendency to caseation. In the tracts of very early infiltration, with no sign of degenerative change, it is a matter of chance whether the section contains a bacillus or not; bacilli are, however, present in these lesions with sufficient frequency to make it clear that they are tubercular in character. It is particularly interesting to note that one or more bacilli may be found in very minute foci, where the only histological abnormality is the presence of a few plasma cells and a few small lymphocytes.

Characters.

The bacilli resemble, morphologically, those found in the livers and lungs. Possibly, not quite so many of the shorter forms are present. No difference is observable between bacilli derived from different viruses.

LYMPHATIC GLANDS.

Histological Changes.

General Characters.

All the lymphatic glands examined proved very much alike microscopically. Various glands were selected for examination, such as the prescapular, bronchial, mediastinal, portal, mesenteric, and popliteal, an endeavour being made to obtain from the material available a piece of tissue which showed early lesions.

All the glands show numerous caseating foci, chiefly in the cortex, with no indication of a boundary zone. No giant cells are present.

Details.

(1) *Lymphocytes and Plasma Cells.*—In sections stained by Pappenheim's method the normal large lymphocytes, the protoplasm of which stains red, are well seen in the medulla; they are also present in the non-tubercular parts of the cortex. At the margin of the tubercles there is generally to be seen a layer of cells, reacting to Pappenheim's stain, some of which are morphologically identical with ordinary large lymphocytes, whilst others exhibit every variety of form found amongst plasma cells. Many of the cells morphologically identical with large lymphocytes are in mitotic division.

(2) *Leucocytes.*—The coarsely granular eosinophil cell which is present in normal lymphatic glands has not been found in any of these tissues. The ordinary multinuclear oxyphil with a fine or more or less indistinct granulation is always present both in the non-tubercular and in the tubercular areas, and is always more abundant in the latter. The number of these cells present is very variable.

(3) *Fibroblasts.*—In the caseating areas the reticular tissue survives rather longer than the lymphocytes, and hence is brought into prominence. At the margin of these areas there is not infrequently found an increase of cells resembling young connective tissue corpuscles. There is no increase of adult fibrous tissue.

(4) *Blood-vessels.*—Blood capillaries, filled with corpuscles, are commonly found within the caseous areas, often with slightly damaged walls. At the periphery of the tubercle there is sometimes found a slight indication of new capillary formation.

(5) *Fibrin.*—Fibrin is present in large quantities in nearly all the tubercular foci, and is often of recent formation.

Presence and Characters of Bacilli.

Numbers.

In almost all the glands examined bacilli are very abundant, more numerous than in the lungs.

Position.

The greatest masses of bacilli are always found within the caseous areas. At the periphery of these areas they are also frequently abundant. They are not infrequently found, in fairly large numbers, in portions of the gland which do not appear histologically tubercular. The relation of the bacilli to the blood capillaries is of interest. Within caseating areas large numbers of bacilli are frequently in close contact with blood capillaries, the contents of which appear to be in process of normal circulation, and sometimes bacilli are seen actually within capillary walls. It is obvious, therefore, that in cases of acute tuberculosis of the lymphatic glands such as these the blood stream is constantly being replenished with large numbers of bacilli. Bacilli are sometimes observed within some of the larger vessels, generally intracellular, and more frequently in desquamated endothelial cells than in multinuclear leucocytes. Within the lymphatic tissue, also, intracellular bacilli are frequently found. They are particularly common within endothelial cells covering the lymph reticulum, and are best seen in the medullary portions of the glands in situations which are not histologically tubercular. Bacilli are also often seen within, or in close apposition to the nuclei of, cells of the type of young connective tissue corpuscles.

Characters.

Morphologically, the bacilli present no differences from those found in the other organs of these animals. No difference is observed between bacilli belonging to different viruses, and it is certainly not the case that the bovine bacilli are either shorter or less frequently beaded than those which are of human origin.

ADDITIONAL MICROSCOPIC DETAILS.

B V.: *Calf* 246.—The liver is less severely affected than usual. In the sections examined, only one small tubercle is present. This is situated beneath the capsule. No tubercle bacilli have been found in it.

B VIII.: *Calf* 254.—This animal died in 19 days. The lesions are of the acute type, but there is rather less tissue destruction than in animals which have lived longer. The granulation of some of the oxyphil leucocytes in the lungs is rather coarser than usual.

B VIII.: *Calf* 256.—A bacillus has been found within a blood-vessel in the liver.

B IX.: *Calf* 244.—This animal died in 19 days. The lesions in the lung, liver, kidney, and popliteal gland are of the usual acute type. In the heart a tubercle was found on one of the chordae tendineae. This contains numerous bacilli, but no oxyphil leucocytes or plasma cells are demonstrable in it. There is a deposit of coagulated material in its centre.

B XI.: *Calf* 232.—Bacilli are somewhat less frequent than usual. The granulation of the oxyphil leucocytes has a tendency to be coarser than usual.

B XI.: *Calf* 234.—Lesions typical of acute infection, but an occasional giant cell in the liver.

B XIII.: *Calf* 272.—The liver is less severely affected than usual. The portion of tissue examined contains one small tubercle in which several giant cells and a few bacilli are present. It is surrounded by fibroblasts. The lungs are severely affected.

B XVII.: *Calf* 258.—This animal died in 18 days. In the lung the lesions are not very advanced, but bacilli are numerous and are generally distributed. The lung is in an acute catarrhal condition.

H 2. *Sp. A.*: *Calf* 569.—The liver is only slightly affected. There are a few small tubercles which contain an occasional giant cell and show a slight tendency to fibrous tissue demarcation. No fibrin is present. In the liver of the companion animal (*Calf* 575) fibrin is abundant.

H 7. *C.M.*: *Calf* 773.—The lesions in the liver are fewer than usual; they are small and contain early fibrin and fairly numerous bacilli. No lesions or bacilli have been found in the specimen of kidney examined.

H 14, *F.S.*: *Calf* 545.—In the liver tubercles are rare; they occasionally contain a giant cell; a few patches of fibrinous deposit are present.

H 16. *J H.* (*Calf* 317): *Calf* 711.—Bacilli very numerous. Early fibrin well shown in lung, liver, and thoracic gland. One giant cell found in a tubercle in the liver.

H 16. *J.H.* (*Calf* 337): *Calf* 789.—The lungs are extensively and acutely infected and contain a good deal of fibrinous deposit. In two respects, however, the lung lesions differ slightly from those found in the majority of the acute cases. (1) Bacilli are less frequent; (2) giant cells are present, and are particularly numerous in the subpleural tubercles. The specimen of liver shows a small, circumscribed tubercle which contains several giant cells and a few bacilli. The kidney contains a similar lesion. A thoracic gland is acutely infected and contains a copious deposit of fibrin. Here again, giant cells are more numerous than is usual in acute cases.

H 17. *Sp. B.* (*Calf* 539): *Calf* 911.—The liver contain some minute, miliary tubercles, but these are less abundant than usual.

H 29. *M.F.*: *Calf* 693.—This animal lived longer than is usual with acute infections. Microscopically, however, the lesions exhibit all the features, including fibrin formation, which are characteristic of acute infection. It is noticeable that many of the lesions present are of

comparatively recent origin and that these contain large numbers of bacilli, whereas some of the lesions which are obviously of older formation contain relatively few bacilli. These observations may be regarded as an indication that the process of the disease became more acute towards the end of the experiment.

H 29. *M.F.*: *Calf* 697.—Some of the tubercles in the liver contain giant cells. This is the only respect in which the lesions differ from those ordinarily found in acute cases.

H 32. *Y.W.* (*Mesent. Gl.*): *Calf* 587.—A few bacilli have been found in the blood vessels. Bacilli are more numerous than usual in the kidney lesions.

H 49. *T.C.* (*Original Material*): *Calf* 787.—This animal lived an unusually long time. Bacilli are particularly numerous in the lungs and kidneys. The lesions in the kidneys are unusually extensive. Early fibrin is well shown in lung, liver, kidney, and thoracic gland.

H 49. *T.C.* (*Original Material*): *Calf* 957.—The lesions in the lung, liver, and thoracic gland are of the usual acute type. The lesions in the kidney are advanced, necrotic, and swarming with bacilli.

H 49. *T.C.* (*Original Material*): *Calf* 959.—In the lung the lesions are of the usual acute type. In the liver miliary tubercles are present, but are less thickly distributed than usual. In a thoracic gland examined, the lesions contain numerous bacilli; they are advanced, acute, and obviously progressive, though there is a tendency to giant cell formation.

H 59. *L.B.*: *Calves* 965 *and* 1063: Calf 965 (from human lung) died in sixty days and shows lesions slightly less acute than the average. Calf 1063 (from human mesenteric gland) died in 28 days; the lesions are of the highly acute type, but show a relatively small amount of tissue destruction.

B. INFECTION NOT FATAL IN NINETY DAYS.

Amongst thirty-five calves inoculated each with 50 mg. of a bovine virus, the infection proved fatal to thirty-four in less than ninety days. The exceptional animal, Calf 240 (B VI.), was killed in apparently good health in fifty-four days. A bull, inoculated with 50 mg. of B V., was killed, also in apparently good health, forty-five days after the inoculation.

Out of 134 calves inoculated each with 50 mg. of culture from 55 different human viruses, 85, representing experiments with 41 viruses, were killed in apparently good health ninety days after the commencement of the experiment. Of these 41 viruses, 37 in no case produced a fatal result. With the remaining 4, certain cultures only failed consistently to produce fatal results.

The most striking feature of the experiments on calves which were killed after ninety days is that, in marked contrast to the cases of fatal infection, the lesions produced were minute, scanty, often difficult to find, and microscopically, were retrogressive in character. In illustration of this fact I give a short histological description of a few typical instances. In reading these descriptions, it must be borne in mind that the pieces of tissue microscoped were not representative of the whole of the organs from which they were taken, but were specially selected, owing to their evidence of disease, from material which, to a large extent, appeared normal.

H 8. *S.C.*: *Calf* 657.—One specimen of the lung contains a minute, circumscribed, caseo-calcareous tubercle in which no bacilli have been found. Another piece of lung contains a suspicious focus which is overlaid with lymphocytes. This is probably a healed tubercle, but no bacilli have been found in it, and its histological characters are not sufficiently definite to justify a positive opinion. In the specimen of the liver, immediately beneath the capsule, there is a small, circumscribed, centrally caseous tubercle in which no bacilli have been found.

H 12 *H.N.*: *Calf* 619.—It was noted at the post-mortem examination that the lungs contained areas of tough, consolidated tissue. Two specimens of these were selected for microscopic examination. They are partially necrotic, densely packed with leucocytes, and not characteristic of tubercular lesions. No tubercle bacilli have been found in them. A poplitcal gland contains a tubercular area with a caseo-calcareous centre. Both in the centre and in the periphery of this area tubercle bacilli are numerous.

H 13. *A.D.* (*Calf* 301): *Calf* 701.—No tubercles have been found in the specimens examined of lung, liver and kidney. A thoracic gland contains some small caseous foci and a few bacilli.

H 23. *J. P.*: *Calf* 625.—A specimen of the lung contains two minute tubercles. One of these has a calcareous centre and contains a giant cell. The other is filled with cells which are mainly lymphocytes. No bacilli have been found, though some material in one of the tubercles might represent fragments of bacilli. In a section of the liver one small tubercle and a few doubtful foci have been found, but no bacilli have been seen. In a thoracic gland there are several slightly caseous areas, some giant cells, and a few tubercle bacilli.

H 30. *E.M.*: *Calf* 593.—Both lungs and liver contain a few minute tubercles, in which no bacilli have been found.

H 30. *E.M.*: *Calf* 599.—Decalcified specimens of the local lesion and the prescapular gland on the same side of the neck show that the tissue, where not calcareous, is densely fibrous and highly necrotic, and that in many places tubercle bacilli are still very numerous.

H 33. *R.T.*: *Calf* 595.—A small caseous tubercle, surrounded with lymphocytes and fibroblasts, is present in the liver. No bacilli have been found in it.

H 35. *C.B.*: *Calf* 605.—In a piece of lung which was selected for examination there is a small tubercle, about two-thirds the diameter of the microscopic field (low power), situated immediately beneath the pleural surface. The rest of the tissue is normal. The tubercle is centrally caseous and is definitely circumscribed. It contains some giant cells and its periphery is infiltrated with small lymphocytes. A few bacilli are present in the tubercle.

H 37. *O.J.*: *Calf* 627.—A specimen of the lung contains a minute fibroid tubercle, in which no bacilli have been found. A thoracic gland shows advanced caseation and contains fairly numerous bacilli.

H 53. *D.H.*: *Calf* 993.—The lungs show many necrotic, densely circumscribed lesions, which contain numerous giant cells, but not many bacilli. In the liver there are several minute foci, some of which contain giant cells; no bacilli have been found. No lesions or bacilli have been found in the specimen of kidney. In a thoracic gland there are caseous, circumscribed lesions containing giant cells; bacilli are scanty.

C. THE PROCESS OF INFECTION.

In the two preceding sections I emphasise the marked differences in the morbid histology of the lesions caused by highly virulent viruses and those caused by slightly virulent viruses, when subcutaneously inoculated into calves in doses of 50 mg. I now proceed to compare in fuller detail the process which is set up when highly virulent viruses are inoculated with this dose with that which is caused by viruses of much feebler virulence.

Early Lesions Produced by Cultures of High Virulence.

Dr. Griffith inoculated Calf 284 with a culture of B XVIII., Calf 292 with a culture of B IV., and Calf 288 with a culture of B XIX., the dose in each case being 50 mg. These three viruses he has found, by other experiments, to be of equally high virulence for the bovine when inoculated subcutaneously in test doses. Calf 284 was killed three days after the inoculation, Calf 292 in eight days, and Calf 288 in fourteen days.

Calf 284 (3 days).

Dissemination of Bacilli.—Sections were cut of the tissues enumerated below. In the case of the lungs, liver, and kidneys, the rule followed in this and in subsequent experiments was that when the whole organ appeared normal the two specimens taken, termed A. and B., were to be selected from widely separated portions of the organ; whenever any portion or the whole of an organ appeared suspicious, one specimen was to be taken from a suspicious area and the other from an apparently normal area or an area which appeared to be the least affected. The statements that bacilli are absent are based on complete examinations, made with the aid of the mechanical stage, of two sections of each portion of lung, liver, and kidney, and three specimens of each of the other tissues. These negative statements are obviously of very much less significance than the positive statements, since the bulk of tissue represented by three sections is extremely small. The prescapular glands referred to in this and in the following experiments are the glands on the same side of the body as the local lesion.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Local Lesion	Present
Lung A	Present
Lung B	Absent
Liver A	Present
Liver B	Present
Kidney A	Absent
Kidney B	Absent
Spleen	Absent
Prescapular Gland	Present
Prepectoral Gland	Present
Bronchial Gland	Present
Mediastinal Gland	Absent
Portal Gland	Absent
Mesenteric Gland	Absent
Popliteal Gland	Absent

Microscopic Details.

Local Lesion.—The tissue examined was taken from the margin, and included some of the underlying muscle. Under a low power view the sections show muscular tissue permeated with numerous areas of cellular infiltration, and replaced here and there by patches of broken-down cell nuclei. Bacilli are abundant, especially in the last mentioned situations. The blood capillaries are numerous and dilated, and bacilli are very frequently found in close proximity to them, occasionally actually within them. Many of the bacilli are intracellular. They are frequently contained within large, mononuclear lymphocytes, and also within large cells possessing pale, oval nuclei; occasionally, but much less frequently, bacilli are found within multi-nuclear leucocytes. Oxyphil leucocytes, all of the finely granular type, are very abundant in all the situations where bacilli are present. Large lymphocytes, some in mitotic division, are also very numerous in the same situations; small lymphocytes are also present, but less common. Pappenheim's stain is unsuccessful, and there is no appearance, in other specimens, of any zones or groups of typical plasma cells.

Lung A.—One or two bacilli have been found with difficulty. They are within blood capillaries and attached to their walls. In some parts of the section there is slight thickening and congestion of the alveolar walls and more multinuclear leucocytes than normal are present. There are no plasma cells.

Lung B.—No bacilli found. Histologically normal.

Liver A.—An occasional bacillus has been found amongst the liver cells, not associated with any histological lesions. There are some small aggregations of leucocytes and lymphocytes in several parts of the section, but no bacilli have been found in these situations. Lesions of this kind are very common in livers and it cannot be assumed, in the absence of bacilli, that they are of tubercular origin. No plasma cells are present.

Liver B.—Only one bacillus has been found. This is present in a minute lesion similar to those described in Liver A.

Kidney A. and B.—No bacilli found. Histologically normal.

Spleen.—No bacilli found. Histologically normal.

Prescapular Gland.—Two pieces of the gland have been examined. Tubercle bacilli are present, chiefly in the cortex, but are not very numerous. There are no lesions associated with them. Some of the bacilli are attached to large lymphocytes. Finely granular oxyphil leucocytes are abundant, especially in the medulla. No coarsely granular oxyphils have been noted. Many lymphocytes and some endothelial cells are in mitotic division.

Prepectoral Gland.—Identical with the prescapular gland, but fewer bacilli.

Bronchial Gland.—Bacilli very rare. No lesions.

Other Lymphatic Glands.—In specimens from one of each of the following lymphatic glands no bacilli have been found, and the only abnormality noted is the presence of an unusual number of multinuclear leucocytes—mediastinal, portal, mesenteric, popliteal.

Calf 292 (8 days).

Dissemination of Bacilli.—The same method of investigation has been employed as with Calf 284.

Tissue.	Presence or absence of Bacilli in the Sections examined.
Local Lesion	Present
Lung A	Present
Lung B	Present
Liver A	Present
Liver B	Present
Spleen	Present
Prescapular Gland	Present
Prepectoral Gland	Present
Bronchial Gland	Present
Mediastinal Gland	Present
Portal Gland	Present
Mesenteric Gland	Absent
Popliteal Gland	Present

Microscopic Details.

Local Lesion.—Enormous masses of bacilli are present in the specimens examined. There are extensive areas of necrotic tissue and fragments of cell nuclei, and at one margin of the sections the bacilli are seen to be infiltrating, and to have partially destroyed, the subcutaneous muscle. The bacilli have evidently multiplied since the date of inoculation, and are often seen growing in clusters round cell nuclei. In the middle of the section there is a large mass of bacilli which is obviously part of the culture inoculated. Comparing these bacilli with bacilli found at the periphery of the section and infiltrating the muscle, it is noted that whereas the former are the typical short, straight bacilli found in serum cultures, the latter are decidedly longer, and are often curved. There are several large blood-channels surrounded with masses of bacilli; it is evident that the walls of these would soon have given way, and that then large numbers of bacilli would at once be thrown into the circulation. Bacilli are frequently present within many varieties of cells, including large lymphocytes, endothelial cells, connective tissue cells, and cells forming the sheath of muscle fibres. Bacilli are not, however, present within typical plasma cells, *i.e.*, cells which have deeply stained eccentric nuclei, marked by basophil protoplasm, and protoplasmic contours which show transitional forms between the spherical and the cylindrical. Oxyphil leucocytes, all of the finely granular variety, are extremely numerous; many of them are of the mononuclear form. Plasma cells are abundant at the margins of the necrotic areas.

Lung A.—General appearance, with small exceptions, normal. In several of the alveoli bacilli are present, and have produced lesions which vary in extent from a slight thickening of the alveolar wall to a tubercle about twice the diameter of an ordinary alveolus. The bacilli present in these situations vary in number from three or four, in the smallest, to 100 or 200 in the largest. In the smallest foci where bacilli are present there are found a few lymphocytes and leucocytes. In the larger foci there are, in addition to a greater number of these lymphocytes and leucocytes, many larger cells with pale oval nuclei, which are very probably derived from the capillary endothelium. There is no evidence of desquamation or proliferation of the alveolar epithelium. Surrounding most of the bronchioles and blood-vessels is a definite ring of typical plasma cells. In the alveolar walls, in many places, are also cells identical in appearance with plasma cells; whether they have migrated from the perivascular and peribronchial connective tissue spaces, or whether they are merely compressed lymphocytes which have escaped from the blood-vessels, cannot readily be decided; it is noteworthy that a considerable number of these cells are in mitotic division. There is no definite aggregation of plasma cells in the tubercles. There are in the tubercles occasional rings of nuclei, evidently belonging to large lymphocytes, which are suggestive of commencing giant cell formation.

Lung B.—Congested and partially collapsed; general thickening of the alveolar walls. A few bacilli occur, sometimes singly and sometimes in groups, but the general condition of this piece of tissue is evidently not associated with the presence of tubercle bacilli. There is a marked leucocytosis. Plasma cells are present as in Lung A.

Liver A.—Bacilli are present within the lobules, but are very rare; they are generally associated with a few lymphocytes and leucocytes. In one situation there is a somewhat larger focus, which may be definitely called a tubercle. It consists of a small aggregation of lymphocytes and leucocytes; some of the former present a confused nuclear mass, suggestive of a giant cell; the parenchymatous cells are partially obliterated, and the endothelial cells are more prominent than normal; a bacillus and the fragments of two other bacilli are present.

Liver B.—Like Liver A. A small tubercular focus is also present within one of Glisson's capsules.

Spleen.—In several situations a few bacilli are present. There are no definite histological lesions.

Prescapular Gland.—Bacilli are very abundant, though less numerous in the medulla than in the cortex. Throughout most of the cortex they are so closely aggregated as to be visible with a low power. The bacilli are evidently multiplying, and though many are adherent to, or within, cells, many are lying free amongst the tissue. Finely granular oxyphils, many of the mononuclear form, are abundant. There are many areas of commencing necrosis and there is also much evidence of increase of the fixed tissue cells, with the formation of imperfect and irregular fibrous tissue. Early fibrin is present.

Prepectoral Gland.—Tubercle bacilli are scattered throughout the gland, but are much less numerous than in the prescapular gland. There is also less tissue destruction. Many of the bacilli are within lymphocytes and endothelial cells.

Bronchial Gland.—Bacilli are present in several parts of the cortex, though their total number is small. They generally occur singly or in pairs. Frequently they are attached to endothelial cells, or to lymphocytes. There are large masses of finely granular oxyphil leucocytes, particularly in the medulla; a few coarsely granular oxyphil leucocytes are also present.

Mediastinal Gland.—Bacilli are fairly numerous. In some situations they are aggregated into groups and have produced small lesions, characterised by commencing necrosis. Elsewhere they are scattered either singly or in twos and threes. They are frequently adherent to cells. Compared with the prescapular gland, the bacilli are not so numerous and the lesions are less advanced.

Portal Gland.—Bacilli are abundant and have produced lesions, some of which are fairly advanced.

Mesenteric Gland.—No bacilli and no histological lesions in the sections examined.

Popliteal Gland. Bacilli are present, but are very rare on the whole. There is, however, one small clump consisting of between thirty and forty. In this last situation there are the nuclear fragments of several leucocytes; otherwise the tissue is histologically normal.

Calf 288 (14 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Local Lesion - - - -	Present
Lung A - - - - -	Present
Lung B - - - - -	Present
Liver A - - -	Present
Liver B - - -	Present
Kidney A - - -	Present
Kidney B - - - -	Present
Spleen - -	Present
Prescapular Gland - - -	Present
Prepectoral Gland - - -	Present
Mediastinal Gland - - -	Present
Portal Gland - - - -	Present
Mesenteric Gland - - -	Present
Popliteal Gland - - -	Present

Microscopic Details.

Local Lesion.—The tissue examined consists of necrotic material interspersed with cellular tracts. In these latter the fibroblastic element is conspicuous. There are also some remains of muscle fibres. Blood vessels are numerous, both in the definitely cellular areas and in the areas which are necrotic. In every situation bacilli are abundant. They are found contiguous to, and sometimes actually within blood vessels. Finely granular oxyphil leucocytes, though abundant in many situations, are distinctly less numerous than in the eight days' specimen. On the other hand there is much more abundant evidence

of an increase of the fixed tissue element. The cells constituting this element undoubtedly have a variety of origins.

The cells forming the muscle sheaths, the endothelium of blood and lymph channels, and the fibrous tissue corpuscles naturally present in subcutaneous tissue, all seem to participate in the new formation. The characteristic of this new formation is that it is loosely and irregularly constructed, and in many places shows signs of commencing necrosis. Many of the spindle-shaped cells react to Pappenheim's stain; characteristic plasma cells are also present, but the differentiation of these cells is not so good as in the eight days' specimen.

Lung A.—Under a low power small, early tubercles can be recognised in every field; generally three or more can be seen in one field. The tubercles evidently originate in the alveolar walls. The largest foci are about four times the size of an ordinary alveolus, and are slightly necrotic in the centre; the smallest are merely slight thickenings of the alveolar walls. All the tubercles are highly vascularised, but the tissue as a whole is not congested; apart from the tubercular foci it is normal. Bacilli are found in all the tubercles, and the largest contain, roughly, from fifty to a hundred. Within the tubercles both leucocytes and lymphocytes are to be found, but, as contrasted with the eight days' lesions, the fixed tissue cells form a much more important constituent of the lesions. With regard to these cells, whilst the cells of the alveolar epithelium have not disappeared, the endothelial cells have definitely increased and become more swollen than normal. Many of these fixed tissue cells contain bacilli, and though the sections, as a whole, show a considerable increase in the total number of bacilli present as compared with the eight days' sections, there is evidently a phagocytic action being exercised by the fixed tissue cells, which is partially restraining the multiplication of bacilli. There are slight traces of fibrin in a few of the tubercles.

Lung B.—The tissue is highly congested. It contains numerous alveolar tubercles similar to those in Lung A. Tubercle bacilli are numerous in these situations; they are also found occasionally in blood-vessels, contained within large lymphocytes. There is an exudate on the pleural surface of the lung and in this situation also bacilli, contained within lymphocytes, are occasionally found. There is a small tubercle projecting on the pleural surface; it contains numerous bacilli and is similar in general character to the tubercles in the interior of the lung; this is interesting owing to the fact that alveolar epithelial cells cannot be regarded as having played any part in its formation. Plasma cells are present in the tubercles but only in small numbers. Traces of fibrin are found in various parts of the section and a few fibrin filaments are seen in the exudate on the pleural surface.

Liver A.—Throughout the tissue, both within and between the lobules, are numerous small tubercular foci which consist of broken down liver cells, endothelial cells, lymphocytes, and leucocytes. They contain bacilli, but only in small numbers. There are a few giant cells. Occasionally a bacillus is found within a liver cell, and occasionally, though not particularly in association with tubercular foci, a liver cell is found in mitotic division. Plasma cells are present but rare. No fibrin is present.

Liver B.—The lesions are like those in Liver A but are much rarer, and tubercle bacilli are fewer.

Kidney A.—In the cortex there are several small lesions. These are either confined to the interstitial tissue, or, if they encroach upon the parenchyma, are evidently of interstitial origin. The lesions contain small numbers of bacilli. No bacilli have been found within renal glomeruli. The lesions are early and show very little necrosis. They contain many plasma cells and small lymphocytes, and also some leucocytes.

Kidney B.—This is less affected than Kidney A., but shows a few slight interstitial lesions, amongst which a bacillus is occasionally to be found.

Spleen.—There are many small patches of commencing necrosis and some giant cells. Bacilli are present in both these situations; they are also frequently found within large mononuclear cells in many situations where there are no histological lesions. Bacilli are fairly well distributed throughout the tissue and are fairly numerous.

Prescapular Gland.—Bacilli are very numerous, forming groups visible with a low power throughout the gland. There is advanced tissue destruction.

Prepectoral Gland.—Identical with the prescapular gland.

Mediastinal Gland.—Bacilli are distributed throughout the gland and are particularly abundant in the cortex, where there are several patches of commencing necrosis. The gland is crowded with oxyphil leucocytes; many of them are of the mononuclear form. In the tubercular foci most of the lymphocytes have disappeared and there is an increase of the endothelial and fibrous elements; this fact is best demonstrated in Pappenheim specimens, where the lymphocytes, when present, give a sharp differential stain.

Portal Gland.—Many groups of bacilli, and also isolated bacilli, are present throughout the gland but mainly in the cortex. The amount of tissue destruction is only slight.

Mesenteric Gland.—Bacilli are fairly numerous, but there is very little tissue destruction. There are several giant cells, each of which contains several bacilli.

Popliteal Gland.—There are a few small groups of bacilli in the cortex, and in these situations the cells show slight necrotic change. Oxyphil leucocytes are moderately numerous. No fibrin is present.

Early Lesions Produced by Cultures of Low Virulence.

Dr. Cobbett inoculated Calves 873, 881, and 869, with cultures of H 26. K. M., a virus which is of low virulence; the dose in each case being 50 mg. These calves were killed 3, 8, and 14 days, respectively, after inoculation. The method of histological investigation followed is in every respect similar to that observed with the three previous animals.

CALF 873 (3 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.	Tissue.	Presence or Absence of Bacilli in the Sections examined.
Local Lesion - - - -	Present	Prescapular Gland - - -	Present
Lung A - - - - -	Absent	Deep Cervical Gland - -	Absent
Lung B - - - - -	Present	Bronchial Gland - - -	Absent
Liver A - - - - -	Present	Mediastinal Gland - - -	Absent
Liver B - - - - -	Present	Portal Gland - - - -	Absent
Kidney A - - - - -	Absent	Mesenteric Gland - - -	Absent
Kidney B - - - - -	Absent	Renal Lymphatic Gland - -	Present
Spleen - - - - -	Absent	Popliteal Gland - - -	Absent

Microscopic Details.

Local Lesion.—The sections examined pass through a mass of the inoculated culture, inflammatory tissue, and subcutaneous muscle. The main histological feature is the enormous mass of oxyphil leucocytes and the remains of leucocytes which surround the inoculated material. It must, of course, be borne in mind that this material acts as an irritant foreign body, and that, therefore, this leucocytic reaction must not necessarily be regarded as a specific reaction against the tubercle bacillus. Whilst the leucocytes have replaced all other histological details in those situations where the aggregation of bacilli is

very dense, in regions where the bacilli are fewer and the process of invasion is at its commencement, there is a definite increase in the fixed tissue elements. The cells lining the spaces between the muscle bundles are swollen, have often assumed a stellate form, and are frequently in mitotic division. Occasionally cells of the capillary endothelium are also found in mitotic division. There is also an invasion of white blood corpuscles in these intermuscular areas; here again the leucocytes are much in excess of the lymphocytes, though some of the latter are present. The leucocytes found in the tissue are of the finely granular type; they are often mononuclear. No plasma cells are present. The blood capillaries are not numerous, nor are they engorged with blood corpuscles. It is noticeable that the bacilli which have travelled some little distance into the tissue are longer than those composing the mass of inoculated culture. Bacilli are very frequently present within fixed tissue cells, and also within leucocytes.

Lung A.—No bacilli found. Patches of slight congestion.

Lung B.—One bacillus found in a blood vessel immediately beneath the pleural covering. Slight subpleural exudation and patches of slight congestion within the lung tissue.

Liver A.—One doubtful bacillus found within a blood vessel.

Liver B.—A few bacilli have been found within the blood-vessels; one is within a portal vein. The bacilli are sometimes attached to blood corpuscles and sometimes enclosed within shadowy material, which may represent broken-down blood corpuscles; one is attached to an endothelial cell on the wall of a hepatic vein, and not far from this bacillus there is a lump of red material, which looks like half digested bacilli, within a lymphocyte.

Kidney A. and B.—No bacilli found.

Spleen.—No bacilli found.

Prescapular Gland.—Under a low power several small suspiciously pale areas were noted. These contain numerous bacilli. Apart from these areas bacilli are rather plentiful in some parts of the cortex, but they are not generally distributed; in some parts of the cortex very few are to be found. Bacilli are rare in the medulla. Several bacilli, all intracellular, are found in the peripheral lymph sinus. The cells which most frequently contain bacilli are lymphocytes and endothelial cells; occasionally one is found within a multinuclear leucocyte. Finely granular oxyphil leucocytes are abundant. A few coarsely granular leucocytes are also present. Some of the lymphocytes have fused together in the process of surrounding tubercle bacilli and present the appearance of a giant cell. No fibrin is present.

Renal Lymphatic Gland.—In one specimen a bacillus has been found within a large mononuclear cell close to the peripheral lymph sinus. Multinuclear leucocytes are more frequent than normally in the gland.

Other Lymphatic Glands.—In specimens from one of each of the following lymphatic glands no bacilli have been found, and the only abnormality noted is the presence of an unusual number of multinuclear leucocytes:—Deep cervical, bronchial, mediastinal, portal, mesenteric, popliteal.

Calf 881 (8 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Local Lesion	Present
Lung A	Absent
Lung B	Absent
Liver A	Absent
Liver B	Absent
Kidney A	Absent
Kidney B	Absent

Calf 881—*cont.*

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Spleen	Present
Prescapular Gland	Present
Deep Cervical Gland	Present
Prepectoral Gland	Present
Bronchial Gland	Present
Mediastinal Gland	Absent
Portal Gland	Absent
Mesenteric Gland	Absent
Popliteal Gland	Absent
Suprarenal Capsule	Absent

MICROSCOPIC DETAILS.

Local Lesion.—The sections pass through a part of the lesion which borders upon the subcutaneous muscle. The portion of the muscle which is nearest to the lesion is infiltrated, while the actual wall of the lesion consists of an imperfectly formed fibrous stroma, in the meshes of which are numerous lymphocytes and leucocytes. As we pass inwards, the number of leucocytes increases, and the tissue becomes more necrotic. On the inner aspect of the lesion the bacilli are very abundant, but their number diminishes with remarkable rapidity as we pass towards the muscle, and hardly any are to be found in immediate proximity to, or between the bundles of, the muscular wall. Oxyphil leucocytes, though plentiful, are much less numerous than in the 3 days' specimen. The nuclei of these leucocytes stain exactly like the nuclei of the leucocytes in the three days' specimen, but their protoplasmic granules are coarser, and have a tendency to escape from the cell body. Lymphocytes are much more numerous than in the 3 days' specimen. No typical plasma cells are demonstrable. Blood capillaries, most of which appear somewhat compressed, are fairly frequent within the lesion. Rudimentary blood capillaries, generally containing a few red corpuscles, are also numerous.

Lung A.—No bacilli have been found. There is an occasional minute focus where the alveolar wall is infiltrated with lymphocytes and a few leucocytes. These might have been caused by any irritant, and, in the absence of bacilli, they cannot be described as tubercles; with these exceptions, the tissue is normal.

Lung B.—No bacilli found. Slightly congested. No lesions definitely tubercular.

Liver A. and B. No bacilli found. No histological evidence of tuberculosis.

Kidney A. and B.—No bacilli found. No histological evidence of tuberculosis.

Spleen.—Two bacilli have been found. No lesions.

Prescapular Gland.—Bacilli are numerous and are generally distributed, but are much less abundant than in the corresponding gland of Calf 292 (the eight days' animal inoculated with a virulent virus). Bacilli are found both lying free and attached to lymphocytes and endothelial cells. Oxyphil leucocytes are fairly numerous, but do not form dense masses. There is hardly any tissue destruction. No fibrin is present.

Deep Cervical Gland.—This gland was removed from the same side of the neck as the prescapular gland. There are no histological tubercles or evidences of tissue destruction, but bacilli, though not so numerous as in the prescapular gland, are distributed throughout the gland in fairly large numbers. Though more plentiful in the cortex than in the medulla, a considerable number are found in the medulla. Bacilli are frequently found attached to endothelial cells covering lymph sinuses. Oxyphil leucocytes are numerous.

Prepectoral Gland.—Similar to the prescapular gland.

Bronchial Gland.—Bacilli are present, but are rare.

Other Glands.—Bacilli have not been discovered in the suprarenal capsule, nor in specimens of the following lymphatic glands:—mediastinal, portal, mesenteric, popliteal.

Calf 869 (14 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Local Lesion - - - -	Present
Lung A - - - -	Present
Lung B - - - - -	Present
Liver A - - - - -	Present
Liver B - - - -	Present
Kidney A - - - - -	Absent
Kidney B - - - - -	Absent
Spleen - - - - -	Present
Prescapular Gland - - -	Present
Prepectoral Gland - - -	Present
Bronchial Gland - - - -	Present
Mediastinal Gland - - -	Present
Portal Gland - - - -	Absent
Mesenteric Gland - - -	Absent
Popliteal Gland - - - -	Absent
Suprarenal Capsule - - -	Absent

MICROSCOPIC DETAILS.

Local Lesion.—The centre of the local lesion was liquid, and the part adjacent to the liquid contents was too soft and friable to be suitable for histological examination. A portion of the wall between the local lesion and the subcutaneous muscle was therefore selected for microscopic investigation. The tissue consists of a fibrous network which penetrates for some little distance into the subcutaneous muscle. The fibrous network is filled with wandering cells, amongst which large lymphocytes, plasma cells, and small lymphocytes are the most numerous. Oxyphil leucocytes are rarer than in the eight days' specimen; frequently, but not invariably, they possess a coarse granulation. Small blood-vessels are present, but are not very numerous; several of them are almost completely obliterated. The bacilli have increased in length, as compared with those in the eight days' lesion. As we pass from the internal aspect of the lesion towards the subjacent muscle, they rapidly decrease in number, and very few are to be found amongst the muscle bundles.

Lung A.—This specimen was taken from a congested patch of lung tissue which was not representative of the lung as a whole. Microscopically, it is congested, partially collapsed, and contains two or three small tubercular foci. These foci are not very clearly marked off from the rest of the tissue. They are slightly necrotic in the centre and contain many leucocytes and some nuclear débris. Each contains two or three bacilli. It is clear that the condition of the tissue as a whole is not due to the presence of tubercle bacilli, and there is no indication that it is likely to become a particularly active focus in the tubercular process. No bacilli have been found in the blood-vessels.

Lung B.—This specimen was taken as representative of the greater portion of the lung tissue, and was normal to the naked eye. Microscopically, the general appearance of the tissue, with the exceptions mentioned below, is normal. (1) There is a small alveolar focus, about the size of an alveolus, which contains four or five bacilli, many leucocytes and broken down leucocytes, and also some lymphocytes and partially disintegrated fixed tissue cells. This focus is some distance from the pleural surface. (2) Another similar focus is present. (3) There are a few other small suspicious foci in which no bacilli have been found. An occasional plasma cell is present both within the tubercles and at the periphery of bronchioles and blood-vessels. No bacilli have been found in the blood-vessels.

Liver A.—There are no histological tubercles. There are some slight abnormalities, consisting of small aggregations of lymphocytes and leucocytes, but in none of these have tubercle bacilli been found. There are a few plasma cells in the interstitial tissue. Within a blood-vessel there are a few tubercle bacilli attached to a mononuclear cell.

Liver B.—One small tubercle is present within a lobule. Its diameter is about one-fifth the diameter of the microscopic field (low power.) One bacillus has been found in this tubercle. The tubercle is centrally necrotic and has a marginal infiltration of lymphocytes and leucocytes.

Kidney A. and B.—No bacilli have been found.

Spleen.—No lesions; a few bacilli are present.

Prescapular Gland.—Bacilli are numerous throughout the cortex, but not so abundant as in the corresponding gland of Calf 288 (the fourteen days' animal inoculated with a virulent virus). As contrasted with the latter, they are not aggregated in sufficient numbers to form groups visible with a low power. The cortex is permeated with extensive tracts of commencing necrosis in which the predominant cells are multinuclear leucocytes and their remains. The majority of the bacilli are relatively long (3 to 4 μ) and curved, but not beaded.

Prepectoral Gland.—Similar to the prescapular gland but rather less tissue destruction.

Bronchial Gland.—A few minute lesions. Bacilli rare.

Mediastinal Gland.—Similar to the bronchial gland.

Other Glands.—Neither bacilli nor tubercles have been found in the suprarenal capsule, nor in specimens of the following lymphatic glands:—portal, mesenteric, popliteal.

LATER PHASES OF THE INFECTIVE PROCESS.

(1.) LESIONS PRODUCED IN NINETY DAYS BY A SLIGHTLY VIRULENT VIRUS.

With the three, eight, and fourteen days' lesions described above, it is interesting to compare the lesions produced in ninety days by the last-mentioned slightly virulent virus.

Calf 871 was inoculated subcutaneously with 50 mg. of culture of H 26. K.M., and was killed ninety-one days afterwards. The following are the details of my microscopic examination.

Local Lesion.—The local lesion was found to consist of liquid, grey material surrounded by a dense fibrous wall. The portion of this wall which has been microscoped, though not so densely calcareous as to necessitate decalcification, consists of a dense fibrous zone surrounding areas of caseo-calcareous material. In the fibrous zone both plasma cells and giant cells are numerous, and there are several imperfectly formed capillary channels, most of which are empty. Tubercle bacilli are present in this zone, though not numerous; they are also found amongst the caseo-calcareous material, but never occur in large groups.

Lung.—In five different specimens neither definite tubercles nor tubercle bacilli have been found.

Liver.—There are many small, suspicious foci, infiltrated with lymphocytes and leucocytes; some of these are strongly suggestive of a tubercular origin, though their histological characters are not quite definite enough to justify their being called tubercles. Four specimens, two from each of two diffrent parts of the liver, have been searched throughout, but no tubercle bacilli have been found.

Kidney.—Specimens, four in all, from two different portions of the kidney have been searched, but neither tubercle bacilli nor lesions suggestive of tuberculosis have been found.

Spleen.—A few minute caseous patches, and a few large giant cells have been found, but no bacilli. (Three specimens searched.)

Prescapular Gland.—This gland, on the same side of the neck as the inoculation, was a dense calcareous mass and decalcification was therefore necessary. In the necrotic part of the tissue bacilli are present in scanty numbers. In the cellular margin giant cells are numerous, but no bacilli have been found.

Prepectoral Gland.—This tissue also required decalcification. Sections closely resemble those of the prescapular gland.

Bronchial Gland.—The gland contains many large tubercles, which are surrounded by fibrous tissue, contain numerous large giant cells, and are caseo-calcareous in the centre. Tubercle bacilli are present in the lesions but are scanty.

Mediastinal Gland.—This gland contains many giant cells, and small caseous areas, but is not so extensively affected as the gland last described. Bacilli are present, but scanty.

Portal Gland.—There are many giant cells and some small tracts of commencing caseation. One bacillus has been found, after searching two specimens.

Mesenteric Gland.—Many tubercles are present. They are slightly caseous, contain numerous giant cells, and show some attempt at fibrous tissue demarcation. Tubercle bacilli are present, but very scanty.

Popliteal Gland.—There are many lesions present which are of the same type as those in the mesenteric gland but more advanced, the centre of some of the tubercles being slightly calcareous. Bacilli are rather more numerous than in the mesenteric gland.

Precrural Gland.—The specimen contains a caseo-calcareous lesion and several smaller lesions which are slightly caseous and contain numerous giant cells. Bacilli are present, but rare.

Suprarenal Capsules.—The specimen contains one large tubercle, which is slightly necrotic in the centre, is partially surrounded by fibroblasts, and contains many giant cells. It is infiltrated with leucocytes and lymphocytes. Tubercle bacilli are present in it but are scanty.

Comparison with the 3, 8, *and* 14 *days' Calves inoculated with H* 26. *K.M.*—In order to compare the dissemination of bacilli, the following table of results obtained with Calf 871 will be useful:—

Tissue.	Presence or Absence of Bacilli in the Sections examined
Local Lesion	Present
Lung	Absent
Liver	Absent
Kidney	Absent
Spleen	Absent
Prescapular Gland	Present
Prepectoral Gland	Present
Bronchial Gland	Present
Mediastinal Gland	Present
Portal Gland	Present
Mesenteric Gland	Present
Popliteal Gland	Present
Precrural Gland	Present
Suprarenal Capsule	Present

Comparison with more virulent viruses.—It is noteworthy that the H 26. K.M. virus is capable of producing in the bovine widely disseminated lesions which, three months after the inoculation, contain tubercle bacilli, and are, histologically, typical of tuberculosis. These lesions are obviously very much less acute, and contain much fewer bacilli than the lesions usually produced by highly virulent viruses.

The virus H 26. K.M. was specially selected for this experiment as being representative of the less virulent human viruses. Calf 865, killed after the same lapse of time, after inoculation with the same dose of the same virus, showed at the post-mortem examination less dissemination of tubercular foci. Out of 136 calves inoculated with viruses of low virulence, Dr. Cobbett finds that 60, or 44·1 per cent. show disseminated foci, sometimes in greater and sometimes in smaller numbers than those found in Calf 871.

(2.) Lesions Produced in ninety days by more Virulent Viruses.

Calves 525, 667, and 905 were inoculated respectively with 50 mg. of H 7. C.M., H 10. B.S., and H 53. D.H. They were killed when apparently in good health 90 days afterwards. It must be pointed out that the viruses H 7. C.M. and H 10. B.S. have been shown by a large number of experiments to be highly virulent for bovines; the virus H 53. D.H., however, is of less virulence for the bovine, having in no case produced a fatal infection. It may, perhaps, be regarded as of intermediate virulence. The results produced on Calves 525 and 667 are exceptional. Calf 525 was a bull-calf six months old, *i.e.*, was a much bigger animal than the calves usually experimented with; the greater resisting powers of this animal must therefore be taken into account. This explanation does not hold, however, for Calf 667, which was of the usual age. The lesions produced in these three animals are of interest for comparison with the lesions produced, in the same length of time, by less virulent viruses. The following are the details of my microscopic examinations.

*H*7. *C.M.: Calf* 525.—The specimen of lung contains five tubercles, each about half the diameter of the microscopic field (low power). The tubercles are surrounded by a dense zone of cells which consist chiefly of small lymphocytes. The centre of most of the tubercles is caseous. A few bacilli are present. The liver contains a few small tubercles which are about the same size as those found in the lungs. They are not caseous, but contain many giant cells. The periphery of the tubercles consists of connective tissue, numerous small lymphocytes, and a few finely granular oxyphil leucocytes. An occasional plasma cell is also found. No tubercle bacilli have been found. A thoracic gland contains caseo-calcareous lesions, in most of which several bacilli are to be found.

H 10. *B.S.: Calf* 667.—The specimen of lung contains 5 tubercles, each about 1 mm. in diameter. The tubercles have a broad peripheral zone of fibroblasts and lymphocytes and contain numerous giant cells. Three of them have caseo-calcareous centres. Bacilli are present in the tubercles in small numbers. In the specimens of liver and kidney no lesions have been found. A mesenteric gland contains a circular, fibro-calcareous nodule in which a few bacilli are present.

H. 53. *D. H.: Calf* 905.—The specimen of lung examined is semi-solid. The consolidated areas are patchy in their distribution and are typical of tuberculosis. Many of them show advanced caseation, and in some there is a minute calcareous deposit in the centre. Some of the foci are circumscribed by a fibrous zone, but in other situations the fibrous element is not conspicuous, and the lesions are ramifying in all directions along the thickened and infiltrated alveolar walls. Bacilli are present in all the lesions and in some foci are numerous. No fibrin is demonstrable. Plasma cells are fairly numerous. Oxyphil leucocytes occur much less frequently than in more acute lesions. Some of them are coarsely granular. A thoracic gland examined shows advanced necrotic lesions with a few imperfectly formed giant cells. Bacilli are numerous. No fibrin is present. In the specimens of liver and kidney no lesions have been found.

(3.) Lesions Produced at Intermediate Periods.

The following three cases illustrate the nature of the tubercular process at different periods after inoculation with 50 mg. of culture. H 46. H.W., which was inoculated into Calf 767, is a human virus of low virulence.

The lesions produced may be regarded as typical. B V. was inoculated into an animal weighing over 9 cwt. (Bull 88) and is a virulent bovine virus. B VI., which was inoculated into Calf 240, aged ten weeks, is also regarded as a virulent virus. All three animals were killed when in apparently good health. The last two results must therefore be regarded as atypical.

H 46. H.W. : Calf 767.—This animal was killed twenty-five days after subcutaneous inoculation, on the right side of the neck, with 50 mg. of culture. The H 46. H.W. virus is known to be only slightly virulent.

Local Lesion.—A portion of the wall was selected for examination. It consists mainly of fibro-caseous material and is nearly, but not completely, avascular. Fairly large numbers of bacilli are present in some of the caseous areas.

Lung.—A few small tubercles are present in each of the specimens examined, which were selected from different parts of the lungs. The tubercles are overrun with leucocytes and lymphocytes; a few of the larger are slightly caseous in the centre. No bacilli have been found in them.

Liver.—In each of two specimens, selected from different parts of the liver, there are a few minute tubercles, the diameter of each being about one-sixth that of the microscopic field (low power). The tubercles are intralobular, surrounded with fibroblasts, and contain groups of lymphocytes and leucocytes. No bacilli have been found in them.

Kidney and Spleen.—The specimens examined proved to be histologically normal. No bacilli have been found in them.

Right Prescapular Gland.—There is advanced tissue destruction with caseo-fibrous degeneration. Bacilli are fairly plentiful, but do not occur in dense groups or clumps; they do not appear to be in active multiplication.

Prepectoral Gland.—There are numerous lesions, which are of the same general type as those in the prescapular gland, but are less necrotic and contain fewer bacilli. The granulation of the ordinary multinuclear leucocytes is rather coarse, and definitely coarsely granular eosinophils are also present.

Thoracic Gland.—The gland examined contains minute caseous foci and some giant cells. A few tubercle bacilli are present.

Portal Gland.—There are giant cells and small foci which are less necrotic than those in the thoracic gland and contain more leucocytes. A few bacilli are present.

Mesenteric and Popliteal Glands.—The glands examined are histologically normal. No bacilli have been found in them.

B V. : Bull 88.—The lung contains numerous large, irregular, partially caseous tubercles which are undergoing fibroid transformation. Bacilli are very scanty. The liver contains fibroid tubercles in which giant cells are numerous, and also more minute foci each of which consists of one or two giant cells surrounded by a cluster of lymphocytes. No bacilli have been found. A section of the kidney shows a tubercle which is of the chronic type and does not contain bacilli. A bronchial gland contains necrotic areas and a few bacilli.

B VI. : Calf 240.—The specimen of lung contains five small tubercles characterised by the presence of many giant cells and a dense infiltration of lymphocytes. A few bacilli have been found in these lesions. No lesions or bacilli have been found in specimens of the liver, kidney, and a precrural gland. A mesenteric gland contains caseous patches and a few bacilli.

II. THE EFFECT OF DOSES OF TEN MILLIGRAMMES.

In 55 experiments a dose of 10 mg. of culture has been inoculated into bovines. In 13 of these experiments the cultures were of bovine origin, and in 42 they were derived from 17 different human viruses found virulent in 50 mg. doses.

A. INFECTION FATAL IN LESS THAN NINETY DAYS.

Amongst the 13 calves inoculated with one or other of the bovine viruses, fatal infection, in less than ninety days, was produced in 9 instances. As representative of these experiments the tissues of the following animals have been microscoped. The list includes an animal fatally infected with a smaller dose.

CALVES FATALLY INFECTED WITH 10 MG. OF A BOVINE VIRUS.

Virus Inoculated.	Number of Calf.	Duration of Experiment in days.
IV. - - - - -	142	60
V. - - - - -	352	40
IX. - - - - -	320	37
XVI. - - - - -	294	46
XVIII. - - -	362	53
" - - - - -	364	47
" - - - - -	366	33
XXIII. - - -	344	48
" - - - - -	346	38

Calf 142 (B. IV.) received 12·5 mg. Calf 294 (B. XVI.) received 5 mg.

Amongst the 42 calves inoculated with bacilli of human origin, a fatal infection was produced in less than ninety days in 30 cases. Of these cases, the following have been selected for microscopic examination:—

CALVES FATALLY INFECTED WITH 10 MG. OF A HUMAN VIRUS.

Culture Inoculated.		Number of Calf.	Duration of Experiment in days.
Virus.	Strain.		
2. Sp. A. - - - - - -	G.P. 534 from Calf 93	889	53
10. B.S. - - - - - -	G.P. 725 from Calf 35	967	48
" - - - - - -	" "	969	48
14. F.S. - - - - - -	G.P. 587 from O.M.*	893	47
16. J.H. - - - - - -	Calf 337	779	63
17. Sp. B. - - - - - -	G.P. 1542 from Calf 553	901	53
" " - - - - -	" "	919	31
19. S.W. - - - - -	Goat 11	921	48
20. F.L. - - - - - -	Calf 213	879	36
28. C.L. - - - - - -	Original Material	813	39
" - - - - - -	Rabbit 102 from O.M.*	819	52
" - - - - - -	" "	877	37
29. M.F. - - - - - -	G.P. 329 from O.M.*	835	40
31. L.F. - - - - - -	Original Material	939	43
" - - - - - -	" "	947	72
32. Y.W. - - - - - -	Human Bronchial Gl.†	851	47
" - - - - - -	" "	855	34
38. J.M. - - - - - -	G.P. 793 from O.M.*	845	36
49. T.C. - - - - - -	Calf 797	929	39
" - - - - - -	"	933	19
59. L.B. - - - - - -	Human Lung	987	49
" - - - - - -	Human Cervical Gl.	1037	41
" - - - - - -	G.P. 1860 from Human Brain	1057	31
" - - - - - -	G.P. 1849 from Human Mesent. Gl.	1077	31

* O.M. = Original Human Material. † Through Rabbit 166.

MICROSCOPIC DETAILS.

With the exceptions mentioned below, the lesions are identical with those described above in the general account of acute lesions produced by the inoculation of 50 mg. doses.

B IV.: *Calf* 142.—The lesions, on the whole, are somewhat less acute than the average. In the lungs, which are very severely affected, the disease is obviously progressive, but there is more evidence than usual of the resisting powers of the tissue. The lesions, instead of

being a disintegrated mass of broken down cellular material, approximate to the characters of organised tubercles. They present numerous, ramifying, caseous tracts, between which are broad, cellular zones containing many giant cells. Bacilli are scattered throughout the tissue in large numbers. In the specimen of liver there are two or three very minute foci, one of which is very probably a tubercle, but no bacilli have been found. In the section of the kidney there is a small tubercle, of the chronic type, in which one bacillus has been found. In a section of a tracheal gland there are no histological lesions, but one bacillus has been found

B XXIII.: Calf 344.—Bacilli are less numerous than usual. Giant cells are occasionally found.

H 2. *Sp. A.* (*Calf* 93): *Calf* 889.—The lesions in the liver are few in number and small. They contain several giant cells and are not of the acute type. Bacilli are present, but scanty. The specimen of the kidney does not show any lesions; no bacilli have been found in it.

H 10. *B.S.* (*Calf* 35): *Calf* 967.—The lesions are advanced and progressive, but bacilli are less numerous than is usual in acute cases.

H 10. *B.S.* (*Calf* 35): *Calf* 969.—In the liver the lesions are not very numerous, for acute cases, and some of them are of the more chronic type, being surrounded with fibroblasts and containing giant cells.

H 14. *F.S.* (*G.P.* 587): *Calf* 893.—The liver is permeated with pale, necrotic tracts which are unlike ordinary tubercular lesions, but bear a close resemblance to the lesions described in the liver of Calf 529, an animal which died 13 days after the intravenous inoculation of 1,000,000,000 bacilli contained in an emulsion of the H 17. Sp. B. virus.* Tubercle bacilli are occasionally found in these areas, but are rare. No other bacteria are present. No lesions or bacilli have been found in the specimen of the kidney.

H 16. *J.H.* (*Calf* 337): *Calf* 779†.—The liver and kidneys are less severely affected than is usual in acute cases.

* See p. 68.

† Calf 783, the companion to this animal, died in 91 days. The lungs are in a condition of advanced, caseating pneumonia, but contain fewer bacilli and more giant cells than are usually met with in very acute infections.

H 17. *Sp. B.* (*Calf* 553): *Calf* 901.—The specimen of liver is not acutely affected. It contains a few small tubercles which are centrally caseous, contain many giant cells, and are bounded by fibrous tissue. Bacilli are moderately numerous within the tubercles.

H 19. *S.W.* (*Goat* 11): *Calf* 921.—The lung is very severely affected, but contains fewer bacilli than usual. The liver, which is in a condition of fatty degeneration, contains rather fewer tubercles than the average. Some of them are extensively caseous. Tubercle bacilli are rare. Caseous tubercles, containing few bacilli, are present in the kidney. In a thoracic gland, which is highly necrotic, bacilli are not very numerous.

H 20. *F.L.* (*Calf* 213): *Calf* 879.—The liver contains numerous small tubercles. In most of these the parenchymatous cells are not completely obliterated; there is an infiltration of lymphocytes, and imperfectly formed giant cells are seen. Tubercle bacilli are present in the tubercles but are not numerous.

H 31. *L.F*: *Calf* 939.—The liver contains numerous miliary tubercles, some of which show a slight fibrous tissue demarcation and contains giant cells; bacilli are fairly numerous in these tubercles and are often found within giant cells. More acute tubercles are also present in the liver.

H 31. *L.F.*: *Calf* 947.—The specimen of lung examined shows much less advanced infection than is usual in fatal cases. The sections show isolated tubercular foci situated amongst normal lung tissue. These foci contain numerous giant cells. The larger foci have a necrotic centre containing particles of calcareous material; they are bounded by a broad zone of fibroblasts and lymphocytes. This zone, however, is frequently contiguous with small, irregular, outlying areas of commencing caseation. The tubercles, therefore, appear to be progressive. Bacilli are scanty. No fibrin is demonstrable. In the specimen of liver there are a few minute lesions which are not sufficiently definite to be recognised as due to the action of tubercle bacilli. No bacilli have been found in them. The thoracic gland examined shows areas of partial caseation with numerous giant cells. Bacilli are scanty, but are frequently found in numbers varying from one to six within giant cells.

B. INFECTION NOT FATAL IN NINETY DAYS.

Out of 13 calves inoculated with 10 mg. of a bovine virus, 4 survived the ninety days' time limit, and were then killed. Out of 42 calves inoculated with 10 mg. of strains of human origin which were known from other experiments to be as highly virulent as one or other of the bovine viruses, 12 were killed immediately after survival of the same time limit. In addition, two calves inoculated with a human virus not fatal in 50 mg. doses also survived the time limit.

The microscopic characters of the lesions found in these animals are as follows.

B V.: *Calf* 354.—The lung contains many isolated tubercles. The tissue surrounding these is normal. The larger tubercles are centrally caseous, and are surrounded by a fibrous zone, but are not completely avascular. The smaller tubercles show commencing caseation and are infiltrated with large numbers of lymphocytes. All the tubercles contain giant cells. Bacilli are very rare. No fibrin is present. Some of the oxyphil leucocytes are coarsely granular. The liver contains small tubercles infiltrated with lymphocytes and containing giant cells. No bacilli have been found in the specimens examined. In the specimen of kidney there is a necrotic nodule containing a few bacilli. In a mesenteric gland examined, there are fibro-caseous tubercles in which giant cells are frequent. Bacilli are scanty.

B XVIII.: *Calf* 360.—The lungs are only slightly affected. They contain small, isolated caseo-calcareous tubercles, which are bounded by a broad zone of fibroblasts and lymphocytes; between the caseous area and the peripheral zone both giant cells and finely granular oxyphil leucocytes are numerous; bacilli are found, in scanty numbers, in the central portions of these tubercles, but not elsewhere; no fibrin is present. The liver contains a few small caseo-calcareous foci which have a dense boundary of fibroblasts and are infiltrated with multinuclear leucocytes; bacilli are very rare; no fibrin is present. In two specimens of the kidney no lesions or bacilli have been found. A portal gland contains caseo-calcareous lesions of the chronic type; bacilli are rare and fibrin is absent. A mesenteric gland contains a few very slightly caseous foci; in these, giant cells are occasionally present; no bacilli have been found. In Calf 360, therefore, the inoculation of 10 mg. of bovine culture has produced a remarkably mild type of infection.

B XXIV.: *Calf* 340.—The portion of lung examined is almost completely solidified, and is undergoing fibroid change. It contains several caseous patches. Some of these are centrally calcareous and are surrounded by a broad fibrous zone. But in many situations caseation is only just commencing, and there is no demarcation into definite tubercles. Giant cells are numerous in all parts of the tissue. There is a dense infiltration of both lymphocytes and leucocytes throughout the tissue; some of the leucocytes have a coarse granulation. Bacilli are present in small numbers; in the caseating tubercles they occur not only in the caseous material but also in the cellular periphery. The liver contains small circumscribed caseous tubercles, some of which have a calcareous centre.

B XXIV.: *Calf* 342.—The lung contains numerous tubercles of the chronic, progressive type; bacilli are scanty. The liver contains many circumscribed tubercles which contain very few bacilli. Some of the older tubercles have a calcareous centre. No fibrin is present in either the lung or liver. Tubercles of a chronic type, but containing bacilli, are found in the kidney. A popliteal gland is extensively affected. Some of the tubercles are centrally calcareous.

H 2. *Sp. A.* (*Calf* 93): *Calf* 883.—The lung is in a catarrhal condition and contains irregular patches of consolidation; some of these are extensive and highly

necrotic ; others are less necrotic but are partially caseous and contain giant cells. Bacilli are scanty in number. No fibrin is demonstrable. In the specimens of liver and kidney no lesions have been found. In a thoracic gland the number of bacilli present is small ; some of the lesions are surrounded by a broad zone of fibrous tissue and are centrally calcareous.

H 14. *F. S.* (*G. P.* 587 *from Original Material*) *: Calf* 895.—The lung shows consolidated areas which have the patchy distribution typical of tubercular pneumonia. In these areas necrosis is not advanced ; they are infiltrated with large numbers of lymphocytes and plasma cells ; oxyphil leucocytes are relatively less numerous. Giant cells are present ; bacilli are scanty. No fibrin is demonstrable. In the liver there are many small, suspicious foci, in which no bacilli have been found. In the kidney there are numerous tubercles. These are only slightly necrotic. They contain many giant cells, in which bacilli are frequently found. A thoracic gland shows lesions of a chronic type, with no fibrin.

H 19. *S.W.* (*Goat* 11) *: Calf* 913.—Histologically the specimen of lung resembles that of Calf 883 (H 2. Sp. A.). Bacilli, however, are very numerous in the caseous and necrotic areas. No fibrin is demonstrable. In the specimens of liver and kidney no definite lesions have been found.

H 20. *F.L.* (*Calf* 213) *: Calf* 867.—No tubercles or tubercle bacilli have been found in the specimens of lung, liver, and kidney. A thoracic gland examined shows small, caseating tubercles containing a few bacilli.

H 28. *C.L. : Calf* 863.—The liver and kidney show some small tubercles in which lymphocytes and fibroblasts predominate. Bacilli are rare in these lesions. A thoracic gland contains extensive caseous tracts ; there is much fibrous tissue formation, and bacilli are rare.

H 28. *C.L. : Calf* 875.—The specimen of lung contains several small tubercles of irregular outline which are densely infiltrated with lymphocytes. The tubercles contain many fibroblasts, but are not surrounded by a definite fibrous layer. Bacilli are scanty. A few suspicious foci have been found in the liver but no bacilli.

H 33. *J.M. : Calf* 827.—The specimen of lung shows pleural thickening, patches of partial collapse and slight infiltration of the alveolar walls, and five or six isolated tubercles. Most of these tubercles are caseo-calcareous in the centre and contain giant cells. In one of the tubercles, amongst the slightly calcareous material of which its centre consists, there is a clump of bacilli which are identical with bacilli grown on serum. In the other tubercles bacilli are either absent or rare. The specimens examined of liver, kidney, and a bronchial gland show no lesions and no bacilli.

H 49. *T.C.* (*Original Material*) *: Calf* 945.—The specimen of lung shows a severe and progressive infection. It is almost completely solid and contains large, confluent areas of advanced caseation, without any demarcation into definite tubercles. Some fibrin is present. Bacilli are very numerous. The liver shows small, caseous, circumscribed tubercles in which bacilli are fairly numerous. Beneath the capsule of the kidney there is a wedge-shaped nodule which is in an advanced stage of caseation and contains numerous bacilli. A thoracic gland is very extensively affected and contains large numbers of bacilli.

H 53. *D.H.**: *Calf* 975.—The lungs contain many small caseous tubercles of the chronic, circumscribed type ; giant cells are plentiful, but bacilli are rare. The liver contains a few small and obviously retrogressive tubercles in which no bacilli have been found. Caseous tubercles are present in the kidneys, but no bacilli have been found in the specimens. A thoracic gland contains caseous lesions, in which giant cells are numerous, but bacilli scanty.

* This virus failed to produce fatal infection in 50 mg. doses.

ANALYSIS OF HISTOLOGICAL RESULTS OBTAINED BY SUBCUTANEOUS INOCULATION OF CULTURES INTO CALVES.

(1.) When inoculated in doses of 50 mg., the strains of bovine origin which have been tested have all proved fatal in less than ninety days. The same result has been obtained with 14 viruses of human origin.* There is a complete histological identity between the lesions produced by these human viruses and the lesions produced by the bovine viruses. The lesions are typical of acute, rapidly progressing tuberculosis.

(2.) Besides the above mentioned 14 human viruses, certain cultures of 4 additional human viruses have produced fatal disease in less than 90 days when inoculated in doses of 50 mg. There is evidence that three out of four of these viruses exhibit, in the cultures referred to, an increase of virulence which is the result of animal passage. It is important to note that the lesions produced by these strains are histologically identical with the lesions produced by the bovine viruses and by those human viruses which were originally of high virulence.

(3.) In these fatally infected animals mentioned in (1) and (2) lesions also occur which are representative of the less acute types of the tubercular process; the characteristics of these lesions are that they are circumscribed by fibroblasts, contain few bacilli, and generally exhibit many giant cells. The histological unity, therefore, between the progressively destructive and the conservative phases of the tubercular morbid process is established by a comparison of the lesions produced in one and the same animal. Both in calves fatally infected with bovine viruses and in calves similarly infected with human viruses, examples of these less acute lesions are to be found.

(4.) In the case of 37 human viruses all the calves inoculated with 50 mg. of culture, in this series of experiments, were killed in apparently good health after the lapse of 90 days. (The few exceptional cases, where intercurrent disease complicated the experiment, are excluded). The same result was obtained in all the experiments made with some of the cultures belonging to the four human viruses mentioned in (2). In all these cases it was found either that general dissemination had not occurred, or that the disseminated lesions which were discovered were small, often few in number, and not actively progressive. These lesions are often definitely recognisable, histologically, as tubercles, and tubercle bacilli are sometimes present in them. But, owing to their small size, rarity, retrogressive or not obviously progressive characters, and owing to the scarcity or absence of tubercle bacilli within them, they stand in very marked contrast to the acute and rapidly progressive lesions found in the fatal cases.

(5.) Further light is thrown on this very wide difference in the pathogenicity of different viruses, when inoculated subcutaneously in 50 mg. doses of culture, by a comparative study of the processes of infection in their earlier stages. The investigation of calves killed at short periods after inoculation shows that the main issue of the experiment is decided in the subcutaneous tissue at the site of inoculation. This tissue is incapable of offering a successful resistance to a highly virulent virus, administered in adequate doses. It is, however, capable of offering against a less virulent virus a resistance which, though not completely successful, is successful to a high degree, and at all events is sufficiently successful to prevent the dissemination of the great proportion of the inoculated material and, consequently, to prevent the reinforcement, from this source, of the bacilli which have been disseminated.

When a virulent virus is inoculated subcutaneously, the tissue at the seat of inoculation offers a vigorous resistance; the bacilli succeed in penetrating the demarcation layer with which the tissue attempts to bar their progress; the resistance on the part of the tissue is continued and again proves unsuccessful; so the process goes on; the resisting tissue which is destroyed from time to time by the invading bacilli remains highly vascular until destruction takes place; consequently frequent opportunities arise for large numbers of bacilli to gain access into the general circulation.

When a less virulent virus is inoculated, the tissue at the seat of inoculation again offers a vigorous resistance; the bacilli are only to a slight degree successful in penetrating the fibrous tissue barrier which is formed against them; this barrier soon becomes complete; the tissue in contact with the bacilli then ceases to be vascularised; consequently, the opportunities for the bacilli to gain access into the general circulation are relatively small, and rapidly diminish. At the site of inoculation the tissue reaction is, within a few weeks, completely successful, and a dense, leathery, fibrous zone is formed which completely debars the entrance of the great mass of the inoculated material into the body. The bulk of the inoculated culture, in fact, is as much external to the body as it would be if it were inclosed within a test-tube.

As a consequence of the different resisting powers of the local lesions, dissemination of bacilli from this source is much more abundant with a virulent than with a less virulent virus, and is maintained for a longer time.

When virulent bacilli are widely disseminated in moderately large numbers, they generally succeed in overcoming the resistance which is offered to them at whatever point in the body they happen to reach; consequently, they speedily multiply, and acute generalised tuberculosis is the result.

When less virulent bacilli are disseminated in very much smaller numbers, the resistance offered to them at whatever point in the body they happen to reach is generally sufficient either to destroy them, or at least to prevent their rapid multiplication; consequently, the tubercular foci which are formed are either obliterated or at least do not readily become fresh starting points for the dissemination of the disease.

(6.) With regard to the small, disseminated tubercles referred to, histological examination confirms the experimental evidence that the bacilli inoculated were of much lower pathogenicity than the bacilli which have produced fatally progressive lesions. After full emphasis has been laid on this fact, attention may be called to further histological details. About some of these tubercles it may be asserted with confidence that the morbid process has been completely overcome. In some of these cases the tissue damage has been extremely slight and the minute lesion has been overrun with lymphocytes; there is no fibrous tissue barrier, no caseation, and no calcification. In other instances, again, the lesion is walled in by a very dense fibrous barrier, enclosed within which there are often found a few calcareous particles. But on the other hand it is not uncommon to find tubercles which, though undoubtedly of the chronic type, cannot be described as "healed." These are centrally caseous and are surrounded by a zone of fibroblasts and other cells. They often contain bacilli. The bacilli, though not very numerous, are sometimes too numerous to justify the assumption that they are merely the relics of bacilli which were actually inoculated and had travelled in the early period of the infection to that particular spot. The original bacilli must have undergone multiplication. Moreover, these caseous foci have certainly expanded since their original formation. There is no proof that, if the animal had been allowed to live, no further expansion would have taken place, nor that the bacilli contained in it would have been annihilated. There is, therefore, the theoretical possibility that such a focus might, later on, have given rise to further dissemination. Taking into account the high resistance of the bovine when living under favourable nutritive conditions and the feeble pathogenicity of the bacilli, such a possibility is very remote. At the same time, the fact that these human bacilli of low virulence have survived for ninety days

* One calf inoculated with a bovine virus and three inoculated with human bacilli, did not succumb to the infection; other calves, however, inoculated with the same strains were fatally infected.

within bovine tissues far remote from the seat of inoculation and are demonstrable within genuine tubercles is evidence of the near relationship between viruses of high and viruses of low virulence for the bovine.

(7.) In the exceptional cases where strains, proved by other experiments to be of high or relatively high virulence, have not produced fatal infection in bovines, the lesions found have often been identical with lesions produced by viruses found experimentally to be of uniformly low virulence; and, when not identical, the lesions have been of a type intermediate in severity between the acute and the chronic.

(8.) In the case of the 10 mg. inoculations with bovine viruses, and with human bacilli which were known to be highly virulent in 50 mg. doses, fatal infection within 90 days was produced in 39 out of 55 experiments. In these fatal cases the lesions are, histologically, of the acute type, closely resembling those produced by 50 mg. of virulent viruses. But, as in the 50 mg. cases, together with these severe lesions, lesions of less severity are found in the same animals; and, in the 10 mg. cases, the occurrence of these lesions of less severity is commoner than in the 50 mg. cases. With regard to the incidence of these variations, there is no difference between the viruses of bovine and those of human origin.

(9.) In the remaining 16 of these 10 mg. experiments the animals survived the 90 days time limit, and 10 of them were reported as apparently "well" when the experiments were terminated. The lesions found in these 16 animals exhibit very great variety in different cases. This variation ranges from lesions which are nearly as severe as those found in the fatal cases to lesions which are small and more or less retrogressive, resembling lesions produced by the inoculation of 50 mg. of viruses of low virulence. Many instances are provided of the definitely progressive type of circumscribed or "chronic" lesion. It is evident that some of the animals would have died from the disease if the experiment had been continued for a longer period. In the incidence of these variations, there is no difference between the effects of the bovine and the effects of the human viruses inoculated.

(10.) The bacilli, in these 50 mg. and 10 mg. experiments, which have produced lesions of the acute, progressive type vary considerably in cultural luxuriance; according to my classification of cultural characters* they fall into one or other of Grades I.–III. The viruses which have consistently failed to produce acute lesions are (with the exception of H 53. D.H., which is classed in Grade III.) of greater cultural luxuriance than the former; they are classed by me in Grades IV.–V.

(11.) The general result of this histological investigation is to bring into emphasis two main points; (1) the marked difference in the severity of the morbid process produced by different viruses; (2) the underlying histological unity, which characterises all these processes, from the mildest to the most severe, as typical of tuberculosis.

* See Part III.

A COMPARISON OF THE INFECTIVE PROCESS IN RABBITS.

ACUTE LESIONS.

On February 17th, 1904, twelve rabbits were inoculated intravenously with a human virus and twelve with a bovine virus, by Drs. Cobbett and Griffith respectively, at their experimental farms. The dose for each rabbit was one milligramme of wet tubercle bacilli. The bovine bacilli were obtained from cultures on dog serum 29 days old, being the eighth generation from a culture isolated from the supramammary gland of Cow 68, which had been infected with the virus known as B III. The human bacilli were obtained from cultures of the same age and grown upon the same batch of dog serum tubes; the cultures were the seventh generation of a strain raised from a guinea-pig which had been inoculated from Calf 5. This calf had been inoculated with the human virus known as H 7. C.M. After inoculation the rabbits were kept at the farms and placed at my disposal for the purpose of histological study.

Course of Experiment.

For the purpose of convenience I make use of the following abbreviations:—

B—a rabbit inoculated with bovine culture.
H—a rabbit inoculated with human culture.

The number prefixed to either of these letters indicates, in days, the duration of the experiment.

Pairs of rabbits, one B and one H, were killed, one, three, five, ten, and fifteen days after inoculation. One B rabbit was killed on the twentieth day. The remaining thirteen rabbits were found dead on the days shown in the following table:—

Number of Rabbits found dead.		Days after inoculation.
B.	H.	
One		16
Three	One	18
	Two	19
	One	20
	Two	22
One		23
One	One	24

POST MORTEM EXAMINATIONS.

LUNGS.

In the right hand column each H specimen is compared with the B specimen, of the same date, which is described in the left hand column. When later cases are compared to earlier, the number prefixed before the letters H or B indicates the duration of the experiment in days.

Bovine.	Human.
One Day.	
About one-third of the surface of one of the lungs is mottled with purple patches which, on section, are found to be superficial only.	Similar; but a larger surface involved. Also a few, more deeply stained, punctiform, subpleural hæmorrhages.
Three Days.	
The changes are of the same type as in 1 B, but extend over a larger surface, and pass rather more deeply into the lung substance.	Similar, but rather more extensive lesions. A few small hæmorrhagic infarcts are found at the tips of the lobes.
Five Days.	
Much more extensively congested than 3 B. The larger of the affected areas extend deep into the lung substance, and exhibit on section a uniform, dark red colour.	Congested areas passing deep into the lung substance are found, but they are mottled rather than uniformly red in appearance.
Ten Days.	
Somewhat congested. Both surface and substance studded with pin-point, translucent, greyish tubercles.	Paler. Both surface and substance packed throughout with slightly larger, grey, translucent, miliary tubercles. The lungs feel less elastic.
Fifteen Days.	
The only marked difference from 10 B is that, in addition to the translucent tubercles, many opaque grey tubercles are seen.	Uniformly pale. More bulky and less elastic. Densely packed with miliary tubercles, which are larger and more uniformly opaque.

Bovine.	Human.
Sixteen Days.	
Identical with 15 B.	
Eighteen Days.	
(1) Tubercles rather larger than in 15 B. (2) Similar. (3) Tubercles rather larger than in (1) and (2), and project rather more prominently on the surface.	Grey miliary tubercles throughout, but tissue less dense than 15 H.
Nineteen Days.	
	(1) Rather more advanced than 18 H, and some of the tubercles larger. (2) Similar to (1).
Twenty Days.	
Lungs semi-solid; generally distributed, early caseation.	Similar.
Twenty-two Days.	
	(1) More extensive solidification than in any previous lung. Individual tubercles larger and more caseous. (2) Similar to (1).
Twenty-three Days.	
Similar to 22 H.	
Twenty-four Days.	
Lungs almost completely solidified.	Similar.

LIVERS.

No tubercular lesions were observed before the tenth day. In both ten days' specimens the livers were observed to be suspiciously pale on section. In both fifteen days' specimens the entire substance of the liver was of a mottled-grey appearance, suggestive of a generalised tubercular infiltration. The later cases exhibited the same condition in a more pronounced degree, and became, progressively, more enlarged and softer. Corresponding to this condition, the surface of the livers was covered with minute, pale, semi-confluent points, but did not exhibit any genuine, discrete, nodular tubercles.

KIDNEYS.

No lesions were seen before the tenth day. On the tenth day, in both rabbits, there were a few minute subcapsular elevations, of circular outline and of the same colour as the rest of the tissue. In both fifteen days' rabbits subcapsular areas of similar outline, but of paler colour, were noted, and also a few minute, pale, miliary tubercles within the renal substance. All the later specimens, with the exception of one H rabbit, contained miliary tubercles, which were present in all parts of the gland, but were generally most conspicuous in the medulla. In no case were large or projecting subcapsular tubercles found.

SPLEENS.

From the tenth day onwards the spleens were enlarged, congested, and of a dull leaden colour. Minute miliary tubercles were noticed on the surface on the eighteenth day, and in all subsequent specimens.

SMEAR PREPARATIONS.

In order to obtain some information as to the spread of the infection, smear preparations were made systematically from the blood of the left auricle and from the marrow of one of the ribs. Smears were also made from lymphatic glands which were notably enlarged. These were only intended as rough observations; they are not sufficiently extensive to constitute a basis for exact comparison.

Blood.

In the B rabbits bacilli were found in the blood of the three days' animal, and in all subsequent cases. In the H rabbits they were first found in the ten days' case, and were present in all subsequent cases.

The bacilli were not numerous (from three to forty in one film); they were almost all intracellular; the cells containing them were generally large and mononuclear.

Bone Marrow.

Bacilli appeared in five days with the B rabbits, and in ten days with the H rabbits. Even on their earliest appearance they were fairly numerous, and often occurred in groups; subsequently their numbers steadily increased and became very large in the later cases. In the sixteen days' B rabbit, the first animal which was found dead, their number was very mueh greater than in either of the fifteen days' cases.

Lymphatic Glands.

Smears were made from swollen mesenteric glands found in both fifteen days' rabbits and in the sixteen days' B rabbit. Tubercle bacilli were abundant in all of them, but much more numerous in the sixteen days' case than in either of the fifteen days'.

COMPLICATIONS.

Several of the rabbits were infected with psorospermosis, which generally involved the liver; but it was always possible to select for histological purposes portions of tissue which contained none of these parasites.

Ten of the rabbits exhibited small, circular foci of a white or greyish colour, which simulated tubercles. These lesions were found most frequently in the caecum or appendix and the terminal inch of the ileum; they were situated immediately beneath the serous coat, but did not involve it. In some of these cases a few pseudo-tubercles were also found immediately beneath the capsule of the liver.

In every instance smear preparations were made from these foci. They all contained short ($1 \cdot 5\mu$), thick bacilli, exhibiting an imperfectly stained gap across the middle, and cocci, which were generally in pairs. None of these organisms were acid-fast. The intestinal lesions contained no tubercle bacilli, but a few tubercle bacilli, in addition to the other organisms, were generally found in the nodules from the liver. Most of the pseudo-tubercles happened to occur in rabbits which were killed during the first ten days; there was therefore no danger of confusing them with genuine tubercles. The occasional pseudo-tubercles which were found in the livers of a few of the later rabbits were also easily distinguishable from the tubercular process, which at that time had assumed the form of a general infiltration.

The incidence of these complications was about equal on the B and H rabbits.

MICROSCOPIC EXAMINATION OF SECTIONS.

LUNGS.

One Day.

B.—Under a low power view there are no well-marked pathological changes in the tissue, but there is a slight thickening of some of the alveolar walls.

Tubercle bacilli are found in small numbers lying in the alveolar walls. They occur either singly or in small groups, and are frequently contained within the cells of the alveolar epithelium and the capillary endothelium. Fragments of bacilli are also noticeable within both epithelial and endothelial cells. When the bacilli occur in groups, multinuclear cells and fragments of nuclei are generally found in close association with them. Bacilli are not found lying free within blood-vessels, nor are any found within the patches of lymphoid tissue adjacent to bronchioles.

There is no evidence of fibrin.

Oxyphil multinuclear leucocytes, with well-marked granulation, are found in fairly large numbers throughout the tissue, and are aggregated in groups in the localities where groups of bacilli are found. There is no infiltration of small lymphocytes. In Pappenheim specimens there are no groups of cells which can be definitely recognised as plasma cells, but a few cells taking a reddish protoplasmic stain are met with in various parts of the tissue. Some of these are found in the nodules of lymphoid tissue and are undoubtedly large lymphocytes; others, generally with oval nuclei, occur at the periphery of bronchioles and the larger blood-vessels. Occasionally a cell with reddish protoplasm is found in the alveolar walls. There is no evidence of newly-formed endothelial cells.

H.—The sections are practically identical with the 1B specimens. The only slight exception is that there are fewer cells which take a reddish protoplasmic stain in Pappenheim specimens.

Three Days.

B.—No more pathological change is noted under a low power than in the one day specimens.

Tubercle bacilli are present, but, in the tissue examined, are less numerous than in the one day specimens. Small groups of bacilli occur and are surrounded with multinuclear leucocytes and nuclear débris. Isolated bacilli are also found within mononuclear cells.

No fibrin is present.

The histological details are the same as in the one-day specimens, with, perhaps, the exception that there is some increase in the small lymphocytes.

H.—The only differences are that tubercle bacilli are more numerous, and there is rather more catarrhal thickening of the alveolar walls.

Five Days.

B.—There is more generalised catarrhal change than in any previous specimen, and there are many small patches consisting of occluded and infiltrated alveoli.

In these patches tubercle bacilli are present in much larger numbers than in any earlier cases. Amongst the walls of alveoli which are still patent, the bacilli are not more diffusely distributed than previously, but, where they are found, they are increased in numbers.

No fibrin is present.

In addition to the multinuclear leucocytes, which are not more numerous than previously, there is a considerable infiltration with small lymphocytes. There are also more cells which react to Pappenheim's stain. These cells are isolated, and many of them are in mitotic division; they are variable in size and shape, and have not the definite characters of typical plasma cells; some of them are small and fusiform, others are about the size of large lymphocytes and have a large spherical nucleus. Whilst it is impossible to form from this specimen a positive opinion as to their origin, the probability appears to be that the former type are modified connective tissue cells, and the latter type modified lymphocytes.

There are many swollen and desquamated alveolar epithelial cells.

H.—The catarrhal change is rather more advanced, and the patches of occluded alveoli are larger.

There is more desquamation of alveolar epithelium, but in other respects the characters of the tissue are the same as in the 1B case.

Ten Days

B.—Small, generally isolated, non-caseous, miliary tubercles are universally distributed throughout the tissue.

Tubercle bacilli are present in these tubercles in such large numbers as to be visible with a low power. In the rest of the tissue bacilli are not universally distributed, but are frequently found within attached or desquamated epithelial cells.

Fibrin filaments are found in some of the blood-vessels and capillaries, but there is no fibrin formation in the tubercles.

The tubercles are packed with oxyphil multinuclear leucocytes; these cells are also more abundant in the rest of the tissue than in previous specimens. The tubercles still contain swollen and desquamated epithelial cells, but there are no signs of any endothelial or "epithelioid" cell proliferation. Small lymphocytes are abundant, and cells which react to Pappenheim's stain are rather more numerous than in the five days' specimens, but still show great variability in size, shape, and intensity of stain.

H.—The lesions are of the same type as the B specimens, but slightly more advanced. The miliary tubercles are a little larger, contain more bacilli, and in some instances are commencing to caseate.

Fifteen Days.

B.—The miliary tubercles are more closely packed than in the ten days' B specimen, and are more necrotic in their centres; they are more irregular in outline, and have more tendency to become confluent. The catarrhal change in the rest of the lung tissue is also more advanced.

The bacilli are again increased in numbers, and are more generally distributed throughout the tissue.

The tissue is swarming with oxyphil multinuclear leucocytes with well-marked granulation. This is the first specimen which gives a typical plasma cell reaction. Unmistakable plasma cells, with bright red protoplasm and a purple, excentric nucleus, are found throughout the tissue in large numbers, and are aggregated in groups round the bronchioles and some of the blood-vessels. Many of them show mitotic figures. In association with the plasma cells are also found many cells which react to Pappenheim's stain, but have less definite characteristics and closely resemble the two types of cells first noted in the five-day specimens. The tubercles contain both swollen epithelial and swollen capillary endothelial cells, but neither show signs of proliferation.

H.—The lung is semi-solid and distinctly more advanced than the B case as regards both the extent of the consolidated foci and the extent of the areas of caseation.

In numbers of bacilli and of oxyphil leucocytes the tissue resembles the B case. In eosin and methylene blue specimens the majority of the latter cells do not exhibit a protoplasmic outline, but consist of a polymorphous nucleus surrounded by a halo of coarse, eosinophil granules. Typical plasma cells are more numerous than in the B specimen, and infiltrate the whole of the tissue, except where it is caseous. Swollen epithelial and endothelial cells are very numerous, and, although they show no signs of active increase, they are so closely packed together that it seems probable that a pathological increase of these cells has previously taken place.

A feature of special interest in this specimen is the cell fusions. (1.) In areas where the alveoli are not completely occluded, desquamated alveolar cells are often found in groups, and in many instances there is complete fusion of their protoplasm, whilst their nuclei are arranged peripherally. Within the protoplasm tubercle bacilli are generally to be found. Passing into completely consolidated zones, similar appearances are seen. The cell fusions here described are irregular rather than circular in outline, and generally the nuclei are not equally distributed at all parts of their periphery. (2.) There are also found, lying free within alveoli which are not completely consolidated, what at first sight appear to be tubes cut transversely and filled with coagulated deposit. On closer examination, the central part of many of these is found to consist of a greatly swollen alveolar cell; this is surrounded on all sides by a layer of smaller cells, which constitute the peripheral, multinuclear portion. Similar appearances are seen within the completely consolidated portions. They are always circular in outline, and have generally a complete, circumferential ring of nuclei. As against the view that some of these may have arisen from occluded blood capillaries, it is to be noted that there are not to be found any corresponding appearances of tubes cut in oblique or longitudinal section. The general conclusion suggested by these observations is that giant cells sometimes arise from a fusion of individual cells, and not necessarily from the nuclear multiplication, without protoplasmic fission, of a cell originally mononuclear.

Eighteen Days.

B. (Found dead.)—Similar to the fifteen days' B case, but rather more advanced. No fibrin.

H. (Found dead.)—Rather less extensive consolidation than in the fifteen days' H case, but not otherwise different. The leucocytes are again very granular, and free granules are scattered throughout the tubercles.

Twenty-three Days.

B. (Found dead.)—Histologically the specimen is very like the fifteen days' H case, but the caseation is more extensive and the number of bacilli present is decidedly greater.

Twenty-four Days.

H. (Found dead.)—More advanced consolidation and caseation, and more tubercle bacilli, than in any previous specimen. As seen under a low power, the entire section is thickly peppered with red points, each of which represents a cluster of tubercle bacilli; and, particularly in the centres of caseation, these clusters are often aggregated into large masses. Both oxyphil leucocytes and plasma cells are still demonstrable, and many discharged granules of the former are found in the caseous centres.

LIVERS.

One Day.

B.—No pathological changes are observable under a low power.

Tubercle bacilli are found in small numbers in the venous capillaries, within liver cells, and in the lymph spaces of the interlobular connective tissue. Sometimes bacilli are associated with small groups of adventitious cells, some of which have round, and some irregular nuclei. The former are recognisable in eosin and methylene blue specimens as small lymphocytes, and the latter as oxyphil, granular leucocytes.

A few cells are found which react to Pappenheim's stain, but do not stain deeply, and have the same characters as those described in early lung specimens. They are generally found in the connective tissue surrounding the larger veins; occasionally a cell taking a similar stain is found within a venous capillary.

H.—Similar; but fewer bacilli and no groups of oxyphil leucocytes.

Three Days.

B.—No lesions are seen under a low power.

There are very few bacilli, and oxyphil leucocytes are also scanty.

In Pappenheim specimens there are found in the perivascular lymph spaces a few cells which take a bright red protoplasmic stain, although their nuclei retain the same spindle-shaped or elongated form which characterises the adjacent normal and undifferentiated connective tissue corpuscles. It seems distinctly probable that these appearances indicate the first stage of transition from a connective tissue corpuscle to a plasma cell.

H.—Under a low power the only abnormalities noted are:—(1) A few small aggregations, both within the lobules and in the connective tissue of the portal canals, of cells with small, deeply staining nuclei; (2) within the lobules, an occasional giant cell.

In addition to occasional isolated bacilli contained within individual cells of the liver parenchyma, several small groups of bacilli are found, and are situated (1) amongst the clusters of small cells, and (2) within the giant cells already mentioned.

In the former situation there are present multinuclear leucocytes, the protoplasm of which is loaded with eosinophil granules, small lymphocytes, cells which have oval or flattened nuclei, and are probably endothelial, and, when these foci are intralobular, some liver cells. The relation of the bacilli to the oxyphil leucocytes is particularly noticeable, because the granules of the latter stain well in Ziehl-Neelsen specimens.

With regard to the giant cells it is interesting to note that each contains a group of bacilli, consisting of from ten to twenty or more, and that this group is surrounded by a complete ring of nuclei. For the following reasons these nuclei may be regarded as derived from the endothelial cells of the venous capillaries. (1) The giant cells are all intralobular, but their nuclei are smaller, stain more deeply, and are more compressed than the nuclei of the parenchymatous cells. (2) They are also unlike the nuclei of the stellate cells, and there is no sign of an increase of the latter. (3) No oxyphil leucocytes are present, and lymphocytes are either absent, or only occur scantily, and external to the giant cells. (4) In some instances imperfectly-formed giant cells, containing each about a dozen bacilli, have been found in actual continuity with the wall of patent and dilated venous capillaries.

It seems probable, therefore, that cells of the capillary endothelium have absorbed minute clumps of the injected bacilli, and, in the attempt to digest them, have undergone nuclear multiplication without segmentation of their protoplasm.

Five Days.

B.—There are many small foci, mostly intralobular, which can hardly be called definite tubercles, but consist of a peripheral part composed of small cells with deeply-staining nuclei, and a central part in which most of the cells are larger and stain less deeply. A few imperfectly-formed giant cells are also seen.

Each of these small lesions, and also each giant cell, contains a group of bacilli. A few bacilli are also found in many parts of the connective tissue of the portal canals. In the last situation the bacilli are mostly intracellular, and have not produced much histological change; in many of the cells only fragments of bacilli have survived. There are also many liver cells and many venous capillaries which contain one or two bacilli, but show an entire absence of tissue reaction.

The histological details of the small lesions first mentioned are as follows:—In their periphery are found a few oxyphil leucocytes, a considerably larger number of small lymphocytes, and an occasional cell which reacts to Pappenheim's stain, though its nucleus is generally larger, more oval or elongated, and less deeply stained than that of a typical plasma cell. Swollen endothelial cells, and amongst them faintly-stained parenchymatous cells, are the chief constituents of the inner part of these foci.

The imperfectly formed giant cells resemble those found in the three days' H. specimen.

The tissue as a whole exhibits only a slight degree of infiltration with oxyphil leucocytes, and there is no evidence of a determination of these or any other type of cells towards the isolated bacilli found within liver cells and venous capillaries. In the perivascular connective tissue there are frequent indications of commencing plasma cell formation.

H.—Small foci are found, about the size of those noted in the B. case, but they are almost completely filled up with small round cells. A few imperfect giant cells are also seen.

As in the B. case, each giant cell contains a cluster of bacilli, but in the other lesions bacilli are much more scanty, and none have been found lying free within venous capillaries or contained within histologically normal liver parenchyma.

Small lymphocytes are the chief constituents of the tubercular foci. Within Glisson's capsules are groups of fairly well-developed plasma cells.

Ten Days.

B.—The entire substance of the liver is uniformly mottled with small, faintly staining, circular, semiconfluent areas which are closely packed together and, in total bulk, occupy from one-third to one-half of the whole tissue. Most of these areas are entirely intralobular, and there is no evidence that they have arisen as an infiltration from the interlobular septa.

In all of these faintly staining areas bacilli are found in large numbers. Throughout the rest of the tissue bacilli are dispersed in small numbers and are generally contained within parenchymatous cells.

No fibrin is present.

Amongst the tissue which is not involved in the definitely tubercular foci both multinuclear leucocytes and small lymphocytes are in excess of the normal, the number of the latter being greater than that of the former. A few cells which retac faintly to Pappenheim's stain, and have the appearance of large lymphocytes, are found in the venous capillaries; the perivascular and peribronchial connective tissues also contain some cells which react to this stain.

There are two striking features about the tubercular foci: (1) They contain a remarkably large number of giant cells in which the nuclei are massed together in the centre of the cell, and are absent from the periphery. (2) Both these giant cells and many other cells, some of which are discrete and others in a condition of partial fusion, exhibit at their periphery numerous protoplasmic elongations which are often continuous with those of neighbouring cells and form a well-marked reticulum.

In addition to giant cells and cell fusions the following types of cells are present in the tubercular foci: (1) Parenchymatous liver cells in a state of partial disintegration. (2) A smaller number of cells with oval or fusiform nuclei, which appear to belong to the remains of the capillary walls. (3) A sprinkling of oxyphil multinuclear leucocytes. (4) A larger number of small lymphocytes.

As bearing upon the probable origin of the giant cells the following features are of interest. In the tubercular areas many partially disintegrated cells are found which are undoubtedly parenchymatous, and which exhibit a tendency to fuse together; these cells have the typical nucleus of parenchymatous cells and, in distinction from lymphocytes and endothelial cells, retain a protoplasmic

stain with eosin in eosin and methylene blue specimens. In some cases, whilst the nuclear characters and the protoplasmic staining properties remain the same, the fusion is so complete as to constitute a single multinucleate cell. If we assume that a cell of this last type then undergoes a multiplication of its nuclei, we have an explanation of the large giant cells which are found containing a mass of nuclei in their centre. The character of the nuclei present is in support of this view; their only difference from the nuclei of ordinary parenchymatous cells is that they are somewhat smaller. Owing to their central position within the cell and to their much lighter staining properties, it is highly improbable that these nuclei are derived from an aggregation of lymphocytes. And, as against the view that these giant cells may be of endothelial origin, it is to be noted that there is no evidence of an active increase of endothelial cells nor of their transformation into cells of epithelioid type.

H.—The general characters of the lesions and histological details are the same as in the B case. Most of the giant cells, however, are of the ordinary type, with their nuclei situated at the periphery. The sections contain an unusually large number of mononuclear cells filled with coarse granules which take a purplish tinge in eosin and methylene blue specimens. These cells do not appear to have any particular association with the tubercular areas.

Fifteen Days.

B.—The lesions are of the same type as in the last two specimens, but rather larger, and are beginning to be definitely necrotic in their centres. Giant cells are less numerous, and none are found with a large mass of centrally placed nuclei.

There is an increase in the number of bacilli.

No fibrin is present.

Plasma cells are more abundant and more characteristically developed than in previous specimens. They are chiefly found in the interlobular tissue, and are sometimes found in large groups surrounding the portal veins. Many of them show mitotic figures. They are also present within or near most of the tubercular foci, but in much smaller numbers.

H.—The tubercular lesions are not quite so extensive or advanced as in the B case, but there is a more copious infiltration with cells, a large number of which are lymphocytes.

Eighteen, Twenty-two and Twenty-four Days.

B and H.—There is no appreciable difference from the fifteen days' specimens.

KIDNEYS.

One Day.

B.—Normal.

H.—One tubercle bacillus has been found within the capillary tuft of a glomerulus, which in other respects is normal.

Three Days.

B.—In one place in the interstitial tissue a few small groups of bacilli are found. The cells associated with these bacilli appear to be chiefly fibroblasts. At this point, and in several other situations where bacilli are not present, there is a very slight increase in the intertubular connective tissue.

H.—No tubercle bacilli have been found. The only difference histologically from the B specimen is that there is a slight increase in the number of oxyphil leucocytes, particularly in the glomerular capillaries.

Five Days.

B.—No bacilli and no histological change.

H.—No bacilli and no interstitial change. Oxyphil leucocytes rather plentiful in the glomeruli.

Ten Days.

B.—In two small patches which exhibit a slight degree of interstitial infiltration tubercle bacilli have been found. One of these patches is immediately beneath the capsule, the other is in the deeper portion of the cortical layer. In these patches there are a few oxyphil leucocytes, but they are much less numerous than the fibroblasts and the small lymphocytes. There are no plasma cells. Some of the tubes in the medulla contain desquamated cells and a coagulated deposit.

H.—One glomerulus contains a clump of bacilli large enough to be visible with a low power. It is situated in the middle of the capillary tuft, which shows some increase of endothelial cells and a slight aggregation of both multinuclear leucocytes and lymphocytes. The capsule of the glomerulus is normal.

Beneath the capsule of the kidney bacilli are present in a few small patches of interstitial infiltration similar to those described in the last B case.

In the medulla there is a larger and more definitely tubercular focus, throughout which bacilli are scattered in moderate abundance. In this patch the renal tubes have been almost entirely obliterated by an invasion of cells, amongst which are many oxyphil multinuclear leucocytes and fibroblasts. It contains capillary channels, from which there appears to be some endothelial proliferation. Lymphocytes are also numerous, and some of the cells present give a faint plasma cell reaction.

Fifteen Days.

B.—Compared with the ten days' B specimen, the patches of interstitial infiltration are more numerous and more extensive, and in some cases have produced a disintegration of the enclosed renal tubes; there is also a marked increase in the number of bacilli. In addition to their presence in the interstitial infiltration, they are found in a good many of the glomerular. These glomeruli, as a rule, show no involvement of their capsules; and in many instances the glomerular tufts would, if it were not for the presence of bacilli, be regarded as normal.

Other details are similar to those of the ten days' B case, but there is a faint indication, in some places, of a plasma cell reaction.

H.—Similar to the last B case, but a more definite plasma cell reaction in the affected areas.

Eighteen Days.

B.—There is an increase in the extent of the lesions, but their general character remains the same. Tubercle bacilli are found abundantly within the interstitial tissue and in the glomeruli, and are also present within the renal parenchymatous cells. In one place a mass of bacilli has been found lying free within Bowman's capsule; but these capsules themselves are rarely involved from within, and therefore there is no evidence of a spread of the infection by direct extension from the glomeruli.

H.—Similar to the B case, but a much better marked plasma cell reaction; a good many of the plasma cells show mitotic figures. The plasma cells occur in large groups in all the more advanced lesions; in all parts of the interstitial tissue between unaltered renal tubules they are found either singly or in chains, and in many of the blood-vessels a considerable number are found. Those found in the blood-vessels can be distinguished from ordinary large lymphocytes by their deeply-stained excentric nucleus, and, in many instances, by their elongated or rectangular form. In the interstitial tissue there are frequently found transitional forms between the apparently ordinary fibroblast which has taken up a red protoplasmic stain and the typical plasma cell. It is impossible to form general conclusions from this specimen alone, but, so far as it goes, it lends support to the view that plasma cells have a twofold origin—(1) from connective tissue corpuscles, (2) from circulating lymphocytes.

Twenty-two and Twenty-four Days.

B and H.—No further changes. In the H specimen the plasma cell reaction is again well marked.

SPLEENS.

Sections of the spleens have been cut and stained by the Ziehl-Neelsen method in order to observe the relation of these organs to the spread of the infection.

In specimens earlier than the fifth day only a few isolated bacilli are found, but on this date there is a decided increase in their numbers.

On the tenth day there is a very marked increase in the number of bacilli; small early tubercles are numerous, and there is a very large number of giant cells.

In subsequent specimens there is a progressive increase, in the number of bacilli, which are present universally and are aggregated into colonies in the numerous areas of commencing necrosis.

Bacilli are more abundant in the B. specimens than in the H.

CONCLUSIONS.

Both the B and the H virus have proved highly virulent to rabbits. This is established both by the macroscopic and microscopic evidence, and by the early dates at which death occurred spontaneously. In the case of the first of these deaths the brain and medulla were examined and were found normal, and there were no embolisms in the pulmonary vessels. There is every reason to believe that in this and in all subsequent cases the termination of life was due to the tubercular infection.

In most respects the histological lesions produced by both viruses were identical in character.

There is some indication, particularly in the lungs, that the H. tissue reaction commenced rather earlier, led to more extensive histological changes, and was, on the whole, slightly more vigorous.

The progressive dissemination of the bacilli by means of the blood stream appears to have taken place rather more rapidly and copiously in the case of the B animals.

ADDITIONAL OBSERVATIONS ON ACUTE LESIONS PRODUCED IN RABBITS.

It has been established in the experimental work that all strains of bacilli, whether of bovine or of human origin, which are virulent for bovines are also highly virulent for rabbits. The lesions in rabbits produced by these virulent strains do not vary very much in character, and the descriptions given above may be taken as typical of them all. This statement is supported by additional detailed histological observations which have been made on the following animals. These specimens do not require separate descriptions.

Rabbits acutely infected with Bovine Bacilli.

Culture inoculated.	Dose.	Mode of inoculation.	Number of Rabbit.	Number of days in which infection proved fatal.
B I. - - - -	·1 mg.	Subcutaneous	83	105
B I. - - - -	·1 mg.	Intravenous	113	21
B II. - - -	1 mg.	Intravenous	152	15
B III. - - -	·1 mg.	Subcutaneous	87	105
B IV. (Chimpanzee 6)	1 mg.	Intraperitoneal	239	20
B V. - - -	1 mg.	Intravenous	145	16
B IX. (Lemur 2) -	1 mg.	Intravenous	535	14
B XI. . - -	1 mg.	Intravenous	163	13
B XI. - - -	·1 mg.	Subcutaneous	564	71

Rabbits acutely infected with Human Bacilli.

Culture Inoculated.	Dose.	Mode of inoculation.	Number of Rabbit.	Number of days in which infection proved fatal.
H 7. C.M. - -	·1 mg.	Intraperitoneal	201	55
H 10. B.S. - -	·1 mg.	Intraperitoneal	296	44
H 10. B.S. - -	10 mg.	Intraperitoneal	298	21
*H 13. A.D. (Calf 301)	37,000,000 bacilli.	Intravenous	38	18
H 14. F.S. - -	1 mg.	Intraperitoneal	278	23
H 19. S.W. - -	1 mg.	Intraperitoneal	235	19
H 29. M.F. - -	1 mg.	Intraperitoneal	274	18
H 38. J.M. - -	1 mg.	Intraperitoneal	250	22

* Tissue emulsion.

The lesions found in the specimens enumerated above resemble the acute lesions in rabbits already described. As in the case of fatally infected bovines, tubercles are sometimes met with in these animals, particularly in the liver and kidney, which are of a more chronic and conservative type. With one exception, the bacilli which have produced these lesions exhibit on artificial media the cultured characters of Grades I.–III., according to my classification, each of these three Grades being represented. The exceptional case is Rabbit 38, which was inoculated intravenously with an emulsion of bacilli from Calf 301 (H 13, A.D.). According to their cultural characters, I place the bacilli of Calf 301 in Grade IV. It is interesting to note that the bacilli of Calf 301, though virulent not only for this animal, but also for calves and rabbits inoculated from it, proved, after residence on artificial culture, to be of greatly reduced virulence for both rabbits and calves.

LESS ACUTE LESIONS PRODUCED IN RABBITS.

I. LESIONS CAUSED BY HUMAN BACILLI.

All those strains of human bacilli which in doses of 50 mg. produce fatal infection in calves in less than 90 days produce a rapidly fatal infection in rabbits. But those strains which have failed to produce severe infection in bovines have also been found to be of lower pathogenicity to the rabbit*. In most of the rabbits inoculated with these last-mentioned strains, lesions, sometimes small and sometimes fairly extensive, have been produced; in a few only has no infection been found.

The following histological details illustrate the nature of the lesions produced by these human viruses or strains which have been found to be of relatively low virulence for the bovine.

H 12. *H.N.*: *Rabbit* 72.—Inoculated intravenously with 1 mg. of culture. Died 179 days afterwards. The specimen of lung is almost completely solidified. It shows large, ramifying tracts of completely necrotic material, with no demarcation into definite tubercles. Irregular patches of less advanced caseation are also present. A few plasma cells are present. Here and there small deposits of fibrin are found. Multinuclear leucocytes

* The Virus H 53, D.H. appears to be an exception to this rule.

with well-marked oxyphil granulation are very abundant. Tubercle bacilli are numerous. There is nothing, histologically, to distinguish this specimen from lesions produced by viruses of high virulence. With virulent viruses, however, the bacilli multiply with much greater rapidity, forming from the second week onwards aggregations which under a low power are visible all over the section as red points. In the lung of Rabbit 72 the bacilli, though present in large numbers in many situations, do not form such dense aggregations. The liver contains many minute miliary tubercles. These are not surrounded by fibroblasts. They show very little caseation, but contain leucocytes, numerous lymphocytes, and cells which are probably modified endothelial cells. Bacilli are present within them, but are not numerous. The damage done to the liver, therefore, is much less than that produced by the intravenous inoculation of highly virulent viruses. The kidney contains fairly large tubercles. These are centrally caseous. They are infiltrated with leucocytes, and are surrounded by a broad cellular zone, which consists mainly of lymphocytes, plasma cells, and fibroblasts. Tubercle bacilli are rather scanty in numbers.

H 17. *Sp. B.* (*Calf* 265)*: Rabbit* 231.—Intraperitoneal inoculation of 1 mg. of culture isolated from Calf 265. Killed 90 days afterwards. The specimen of lung is in a semi-solid condition, owing to the presence of innumerable tubercles. These tubercles are centrally caseous, irregular in outline, and frequently confluent. The tubercles are not surrounded by fibrous tissue, but their periphery, and the adjacent tissue, is characterised by a dense cellular infiltration. They are evidently progressive. The fixed tissue elements found in the tubercles consist partly of cells resembling epithelial cells, and partly of cells with large, pale, oval nuclei, which are probably of endothelial origin. In the cellular infiltration oxyphil leucocytes, small lymphocytes, and plasma cells take part. The oxyphil leucocytes are very numerous in all parts of the tissue; lymphocytes are, relatively, less frequent; there are many groups of plasma cells at the periphery of consolidated areas. In the majority of the foci bacilli are scanty, but here and there, particularly in the more advanced lesions, large groups of bacilli are present. The liver contains very numerous minute tubercles. These are not caseous, but exhibit cell fusions and sometimes giant cells. Both oxyphil leucocytes and small lymphocytes are present in these foci; the latter are much more numerous than the former. The liver parenchyma in these areas has been replaced by cells with large, pale, frequently oval nuclei. No tubercle bacilli have been found. In a specimen of the kidney and in a mesenteric gland neither lesions nor bacilli have been found.

H 23. *J.P.: Rabbit* 241.—Killed 90 days after intraperitoneal inoculation with 1 mg. of culture. The specimen of lung shows an irregular, slightly caseous patch of consolidation in which tubercle bacilli are present but scanty. This area is densely infiltrated with lymphocytes and leucocytes. No fibrin is present. A few suspicious foci have been found in the liver, but they are not sufficiently definite to be called tubercles. No bacilli have been found in them. No bacilli or lesions have been found in specimens of the kidney nor in a mesenteric gland.

H 27. *B.D.: Rabbit* 304.—Killed 89 days after intraperitoneal inoculation with 1 mg. of culture. In the lung there are many small tubercles which are slightly necrotic in the centre. Tubercle bacilli are present in these foci, but are rare. In the specimen of liver there are a few suspicious foci, in which no bacilli have been found. The kidney shows definite tubercles both in the cortex and in the medulla. They are surrounded by a well-marked cellular area in which plasma cells are abundant. One of the tubercles contains in its centre, which is caseous, large numbers of bacilli. A mesenteric gland contains some tubercles which are filled with caseous material and nuclear débris. No bacilli have been found in these lesions.

H 30. *E.M.: Rabbit* 212.—Killed 88 days after intraperitoneal inoculation with 1 mg. of culture. The lung contains many tubercles of a slowly progressive type. These tubercles are centrally caseous; their periphery and the adjacent tissue is densely infiltrated with lymphocytes. Their outline is irregular and there is no definite zone of fibroblasts. In some of the caseous patches bacilli are fairly numerous, *e.g.*, 40 or more occur in one microscopic field. The liver shows a caseous tubercle beneath the capsule in which bacilli are numerous. The specimen of kidney contains a large lesion which appears to be in process of repair. It is not caseous but contains many fibroblasts. In its interior there are several bacilli. In a mesenteric gland no lesions have been found.

H 30. *E.M.: Rabbit* 215.—Killed 88 days after intraperitoneal inoculation with 1 mg. of culture. The lung contains some isolated caseous tubercles in which bacilli are present in small numbers. In the liver a small tubercle surrounded with fibroblasts and not containing any bacilli has been found. No lesions have been found in specimens of the kidney and a mesenteric gland.

H 33. *R.T.: Rabbit* 217.—Killed 90 days after intraperitoneal inoculation with 1 mg. of culture. Beneath the pleural surface of the lung there is a tubercle in the early stage of caseation. It contains a few bacilli, and is infiltrated with coarsely granular oxyphil leucocytes. These cells are also numerous in the rest of the tissue. In a specimen of the kidney there are no lesions. In a mesenteric gland there are caseous foci containing large numbers of bacilli.

H 33. *R.T.: Rabbit* 219.—Died 69 days after intraperitoneal inoculation with 50 mg. of culture. The lung is in a condition of patchy consolidation. The consolidated areas are slightly caseous; they are filled with swollen epithelial cells, and are densely infiltrated with oxyphil leucocytes. Tubercle bacilli are present in all parts of the tissue, and are numerous in some of the caseous areas. They are often contained within desquamated epithelial cells. No fibrin is demonstrable. The specimen of liver shows no lesions. In the kidney there are many small, slightly caseous tubercles containing numerous bacilli. In a posterior sternal gland the tissue is almost completely replaced by caseous material which contains numerous bacilli.

H 37. *O.J.: Rabbit* 238.—Killed 90 days after intraperitoneal inoculation with 1 mg. of culture. The lung exhibits patches of consolidation similar, histologically, to those found in the lung of Rabbit 219. But these patches are fewer in number and contain fewer bacilli. Specimens of the liver and kidney show no lesions.

H 37. *O.J.: Rabbit* 240.—Killed 90 days after intraperitoneal inoculation with 50 mg. of culture. The lung is in a similar condition to that of Rabbit 219.

H 41. *A.S.: Rabbit* 320.—Killed 89 days after intraperitoneal inoculation with 1 mg. of culture. In the lung, kidney, and a mesenteric gland no lesions have been found. In the liver, immediately beneath the capsule, there is a caseous nodule circumscribed by fibrous tissue and containing large numbers of bacilli. From the appearance of this nodule it is probable that it was formed by a deposit of the inoculated bacilli upon the capsule of the liver. The fact that it is surrounded by a fibrous barrier and that the adjacent liver tissue is normal illustrate very well the high degree of resistance offered by the liver to these bacilli.

H 43. *F.F.: Rabbit* 328.—Killed 89 days after intraperitoneal inoculation with 1 mg. of culture. In specimens of the lung, liver, and a mesenteric gland no definite lesions have been found. In the medullary portion of the kidney there are several tubercles which are only slightly necrotic, and appear to be in process of repair. They contain very few bacilli.

H 44. *D.C.: Rabbit* 346.—Died 90 days after intraperitoneal inoculation with 1 mg. of culture. The lung contains many small, slightly caseous tubercles in which bacilli are present, but very scanty. The liver shows some minute tubercles in which no bacilli have been found. No lesions have been found in the kidney. In a thoracic gland small caseous foci are present and contain a few bacilli.

II. LESIONS CAUSED BY BOVINE BACILLI.

All the viruses of bovine origin are highly virulent for the rabbit. Instances where the bacilli inoculated did not produce severe infection are very rare and appear to be accidental. For example, Rabbit 570, inoculated subcutaneously with ·001 mg. of B XI. and killed 45 days afterwards shows very little sign of infection. In a bronchial gland there is a minute caseous focus containing one bacillus; in the lung I have found one or two suspicious patches, but no definite tubercles and no bacilli; the liver and kidney are normal. But Rabbit 567, inoculated subcutaneously with the same dose of the same virus and killed after the same interval, shows much more disease. The lungs contain numerous caseous tubercles which are closely set together and are surrounded by a very dense cellular zone. In this zone giant cells are found and there are many fibroblasts. Bacilli are numerous in some of the caseous areas, and are also present in the rest of the tissue. Many tubercles are present in the liver. These are all of the more chronic type; they are surrounded by a broad cellular zone which shows a vigorous attempt at fibrous tissue demarcation; giant cells are numerous; bacilli are scanty. A specimen of kidney shows no lesions. A bronchial gland is acutely infected.

Rabbit 565, inoculated subcutaneously with ten times as big a dose as Rabbit 567, the same virus being employed, and killed after the same interval, shows lesions of the same type as those found in Rabbit 567, but the lesions are much less numerous and the individual lesions are less extensive. A subcutaneous inoculation of the same virus was also given to Rabbit 563 with a dose of ·1 mg., *i.e.*, 100 times as large a dose as that given to Rabbit 567. Comparing these two rabbits, the main differences noted are that in the lung of Rabbit 563 the lesions are rather more advanced, but the liver of this animal is much less extensively affected.

Rabbit 225, inoculated subcutaneously with 1 mg. of culture of B I., may be regarded as an instance where the disease followed a less acute course than usual. This animal was killed after survival for 114 days. The lungs are in very much the same condition as those of Rabbit 563. The specimen of liver shows two minute, non-caseous tubercles, in which no bacilli have been found. The kidney shows many large, caseous tubercles in which bacilli are abundant. In a mesenteric gland which has been examined there is no evidence of tuberculosis.

It has been shown that rabbits are not readily susceptible to highly acute infection by the alimentary tract. For example, Rabbit 89 was fed for thirty-one days with the tuberculous milk of Cow 172 (B IV)., and was killed 70 days after the commencement of the experiment. The lung shows small isolated tubercles which are only slightly caseous, are infiltrated with lymphocytes and leucocytes, and contain very few bacilli. These tubercles are identical with the minimal lesions produced in the lungs in some of the inoculation experiments with viruses of human origin. The kidney shows no lesions. A mesenteric gland shows large caseous areas in which bacilli are fairly numerous.

III. ANALYSIS OF RESULTS.

The main results brought out by the study of these less acute lesions are:—

(1.) Those bacilli of human origin which are of low virulence for the bovine are also, with perhaps one exception, of relatively low virulence for the rabbit.

(2.) These bacilli of low virulence are, however, capable of producing tuberculosis in the rabbit. The morbid process set up, though of much less severity, and, when progressive, of much slower course, is the same in kind as the morbid process set up by more virulent bacilli.

These results are well illustrated by the case of Rabbit 72. This animal was inoculated intravenously with 1 mg. of H 12. H.N., a virus known to be of low virulence for bovines. Fatal infection was produced in the rabbit, but the disease ran a course of 179 days. A similar dose of a virus highly virulent to the bovine would have produced death in from two to three weeks. Attention may also be called to Rabbit 231, which was inoculated intraperitoneally with 1 mg. of culture from Calf 265 (H 17. Sp. B.), and killed 90 days afterwards. Evidence of progressive disease in the lungs has been found, although this strain of bacilli is of low virulence to the bovine.

SECTION II.

LESIONS PRODUCED UNDER VARIOUS EXPERIMENTAL CONDITIONS.

EXPERIMENTS ON BOVINES.

The Method of Investigation.

The following is an explanation of the method pursued in the work recorded in this part of my report.

Selection of Material.

Tissues have been selected for histological examination with the object of studying the characters of the lesions produced by the various bovine and human viruses when introduced into the body of the bovine. The number of bovines used for experiment has been so great that it has been impossible to examine the tissues of more than a relatively small number of them, but an effort has been made to examine a number of cases sufficiently great to be fairly representative of all the important results which have arisen during this part of the experimental work. I have considered that the organs most useful for examination were :—The udder (when infected), the lungs, the liver, the kidneys, and the lymphatic glands.

Method of Recording Details.

The tissues have been examined and reported on as soon as convenient after the conclusion of the particular experiments to which they belonged. The characters of each tissue have been made an independent study and have been recorded without reference to any theory or hypothesis of what lesions viruses of one origin or another might be expected to produce ; in a great many instances the records were made at an early stage of the experiment, before sufficient evidence had accumulated to warrant a definite opinion as to the degree of virulence of the virus or strain in question. These results have now, for the purpose of convenient reference, been put together, in the numerical order of the viruses and animals to which they belong, and a brief summary, giving the gist, or some prominent feature of the details, has been placed beneath each.

The Diversity of Experimental Conditions.

The animals from which it was necessary for me to obtain my specimens had been placed under a very great variety of experimental conditions. It is necessary to consider these conditions in so far as they may affect the interpretation of my histological results.

(1.) The material inoculated was, except in a few cases where various doses of culture have been used, an emulsion of tubercular lesions derived from experimental animals, or from the human body, or from bovines killed at the slaughter house. My experience in examining these emulsions, which were sent to me for the purpose of estimating the number of bacilli they contained in a given sample, shows that they differed widely in character. Sometimes their solid constituents were composed almost entirely of caseo-calcareous material, together with a certain number of bacilli, generally, though not always, a scanty number ; and the lesions from which these emulsions had been made were often of long standing. On the other hand, emulsions were often examined in which bacilli were present in enormous numbers, and were obviously in a very active state of multiplication ; these emulsions were generally made from tissues where the lesions were of more recent date. It is clear from my observations that at the time of inoculation the bacilli in some cases were and in some cases were not, in a condition of active multiplication ; their condition in this respect may, in some instances, have had an influence on the effect which the inoculation produced. In association with this consideration may be placed the fact, established by bacteriological experience, that if two subcultures are made on to identical media, the one from a poorly growing and the other from a better growing culture of the same strain, the latter subculture will grow better than the former.

(2.) The doses inoculated have varied within very wide limits.

(3.) The majority of the animals have been inoculated by the subcutaneous method. In the case of certain others the method adopted has been intramammary, intravenous, or intraperitoneal. In a few cases the bacilli have been introduced by feeding.

(4.) The animals have not all been of the same age, and it has been found that the older animals possess a higher power of resistance than the younger.

(5.) The period of duration of the experiments has been very irregular.

Interpretation of Results.

This diversity of experimental conditions has brought to light many interesting histological features which might not otherwise have been observed. At the same time it has rendered the interpretation of histological results a complicated task. I have therefore found it necessary, after recording my histological results in detail, to submit them to a brief analysis and discussion.* For this purpose the details fall into three main divisions. (1) Lesions produced in the udder. The results produced by the intramammary inoculations form very useful examples of local lesions ; they exhibit the different effects of doses possessing different degrees of virulence in much more detail than do the local lesions formed after subcutaneous inoculations. Lesions formed in the udder after subcutaneous inoculation in the neck also possess an interest in relation to the question of the spread of the disease to the udder in animals spontaneously infected with internal tuberculosis. (2) Lesions produced in the lungs. These lesions are taken by themselves, because the lungs are by far the most important organ. It may be said that, in all but a few exceptional cases, the degree of success or failure in resisting the disease which the animal exhibits is directly proportionate to the extent and severity of the tubercular process found in the lungs. (3) Lesions produced in other internal organs. As examples of these organs I take the liver, kidneys, and lymphatic glands. The distribution and character of the disease in these situations, taken together, throw additional light on the capacity of the different viruses and strains inoculated for producing general dissemination of the disease.

Conclusions and Comparisons.

Based on the above analysis, I formulate certain conclusions † as to the nature of the morbid processes which have been caused by the different viruses, and then compare these viruses according to the resemblances and differences in the histological character of the lesions produced.

* See p. 96. † See p. 102.

THE HISTOLOGICAL DETAILS.

I. EXPERIMENTAL TUBERCULOSIS OF THE UDDER.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Cow 18.

Cow 18 received an intramammary injection of bacilli contained in an emulsion of the organs of eight guinea-pigs which had been inoculated with the original material of the virus B I. Tho inoculation was made into the left fore and right hind quarters of the udder. The animal was killed 35 days afterwards.

In the non-inoculated quarters sections taken from several areas show that the greater portion of the tissue is normal, but occasionally small foci are found in which one or more alveoli have been obliterated. There is also a slight degree of infiltration in the interlobular and interalveolar tissue. Within these areas of infiltration and surrounding the foci where the alveolar structure has been obliterated, plasma cells are numerous. Oxyphil leucocytes are not numerous in the interstitial tissue, but often occur in groups within otherwise normal alveoli. The foci where the alveolar structure has been damaged contain broken down parenchymatous cells and leucocytes. Tubercle bacilli are present in these situations and are also found occasionally in the interstitial tissue. Many of the tubercles contain a deposit of recently-formed fibrin.

The inoculated quarters exhibit advanced disease. In many sections there is little or no recognisable mammary tissue left, and the general appearance is one of advanced necrotic change without any demarcation into definite areas. There is no indication of fibroid repair or calcareous change, and there are no giant cells. Blood capillaries are frequently met with, except in the central parts of the necrotic areas, and are distended with red corpuscles, which generally have retained their normal contour. In the transitional areas between the extensively diseased and the relatively normal portions of the udder, there is more evidence of fibroid change. The interstitial tissue is greatly increased, owing to the presence of many fibroblasts, plasma cells, and leucocytes, but many irregular, caseous patches are present which increase in number and size as we approach the areas showing macroscopic evidence of advanced infection. It was noted at the *post-mortem* examination that the milk sinuses near the nipples of the inoculated quarters were covered with tubercular granulations. Microscopically, these sinuses exhibit the same type of acute and diffuse tubercular infection as the mammary tissue. Immediately beneath the epithelium lining these sinuses dilated capillaries filled with red corpuscles are often found.

Throughout the inoculated quarters tubercle bacilli are present everywhere in large numbers and often form red masses conspicuous under a low magnification. There is much destruction of elastic tissue, though in areas even of advanced necrosis some elastic fibres have survived, and appear as isolated strands running in straight lines across the field. Fibrin is not found in the central parts of the older lesions, but is generally demonstrable at their periphery. No calcareous deposits have been noticed.

The majority of the bacilli are straight or nearly straight, and vary in length from $1 \cdot 5$ to 3μ. They are often imperfectly beaded. Longer bacilli, from 3 to 4μ in length, are also found ; these are generally curved and are often definitely beaded.

Summary.

Acute, progressive infection, with rapid tissue destruction and great multiplication of the bacilli.

VIRUS.—B I.

Animal Inoculated.—Cow 40.

Cow 40 received an intramammary injection, into the left fore and right hind quarters, of "5 c.c. of an emulsion of pure culture." The culture was a primary culture obtained from G.P. 14, which had been inoculated with the original material, and was 57 days old. Cow 40 was killed 92 days later.

The specimens taken from the non-inoculated quarters show no evidence of tuberculosis.

The portions of tissue removed from the infected parts of this animal's udder were extremely tough and gritty, and therefore required decalcification. Microscopically, the predominant feature of sections taken from various parts is the dense fibroid and calcareous degeneration. Broad strands of this material, calcareous centrally and fibrous peripherally, ramify in all directions. Another striking appearance, with a low magnification, is the large number of giant cells, from 70 to 80μ in diameter, with an approximately circular outline and a more or less even distribution of nuclei round the entire cell periphery. Examined under a higher power, these giant cells do not appear to have been associated either with secretory cells or with any other tubular elements, but to have arisen by a fusion of cells rich in protoplasm and of an endothelial type in places where the glandular tissue has completely disappeared. Cut off here and there at the margins of the fibro-calcareous patches, remains of the mammary tubules are to be found. Very few blood-vessels are present. No tubercle bacilli have been found in the parts histologically normal. An occasional bacillus has been found in the less necrotic parts of the infected tissue, but the total number present appears to be extremely small, and many sections have been searched without the discovery of a single bacillus.

Summary.

The lesions are extensive, but are densely fibro-calcareous in character and contain very few bacilli. The great age of the culture used for inoculation must be borne in mind.

VIRUS.—B I.

Animals Inoculated.—Cows 44 and 64.

Cow 44 received an intramammary inoculation of 1,000,000 bacilli into the left fore and a similar dose into the right hind quarter. The bacilli were contained in an emulsion of the prescapular gland of Calf 126. Cow 44 was killed 41 days after inoculation.

Cow 64 received an intramammary inoculation, into the left fore and right hind quarters, of milk from the infected quarters of Cow 44. The milk inoculated into each quarter was estimated to contain 500,000 bacilli. Cow 64 died 48 days after the inoculation.

The udders of these two animals present similar histological features. In the inoculated quarters the tissue is replaced by caseous and necrotic material which extends diffusely in all directions, without demarcation into definite tubercles. No calcareous deposits have been found. Bacilli, though numerous in both udders, are much more abundant in the inoculated quarters of Cow 64. The amount of tissue destruction also appears to be greater in this animal.

The specimens of the non-inoculated quarters of Cow 44 have been found to be normal. In one portion of the non-inoculated quarter of Cow 64 there is marked interstitial mastitis, but I have not found either tubercle bacilli or definite tubercles.

Summary.

Progressive, caseating tuberculosis; rather more acute in the udder of Cow 64.

VIRUS.—B I.

Animal Inoculated.—Cow 74.

Cow 74 received an intramammary inoculation, into the left fore and right hind quarters, with an emulsion of the udder of Cow 40, which showed lesions of the fibro-calcareous type and contained very few bacilli. Cow 74 died 299 days after inoculation.

In the non-inoculated quarters there is found a marked increase in the fibrous tissue elements both between the lobules and between individual alveoli. The interstitial tissue is highly vascular. Within many of the alveoli are small calcareous concretions. There is no histological evidence of tubercular infection. The elastic tissue is abundant; many of the fibres are particularly thick.

An occasional plasma cell is found in the interstitial tissue.

Oxyphil leucocytes are present in the interstitial tissue, but are very scanty; their granulation is indistinct. Some of the smaller calcareous deposits within the alveoli are seen to be formed as concretions round desquamated epithelial cells.

Tubercle bacilli occur in small numbers in both these quarters. They are found lying free in the interlobular interstitial tissue and between the fat vacuoles. The situations in which they occur are histologically normal.

In the inoculated quarters the following details have been observed.

Left Fore.

The Tissue near the Nipple.

General Histological Characters.—The sections prepared contain large circular tubercles in a fairly advanced stage of caseation and with small calcareous foci in their centre. Blood capillaries are found in the parts of these nodules which are not caseous. The nodules are surrounded with a broad fibrous tissue zone. The rest of the tissue is highly vascular, and contains groups of normal mammary alveoli side by side with areas in which the alveoli have been partially replaced by tubercular infiltration. These tubercular areas appear to have advanced by way of the interstitial tissue. They are irregular in outline, and have no fibrous tissue boundaries; they are partially caseous, and contain broken down red blood corpuscles and nuclear débris.

Fibrin.—In the large circumscribed nodules no fibrin is found. In the areas of irregular tubercular infiltration there is a good deal of material which stains black by Kochel's method. Much of this consists of broken down and intact red blood corpuscles, but definite fibrin fibrils are also found, though not in large quantities.

Minute Characters of Cells.—Round the margins of the circumscribed nodules plasma cells, passing into fibroblasts, are found. In the rest of the tissue plasma cells are numerous both in the definitely tubercular areas, and infiltrating the interstitial tissue between unaltered glandular alveoli. Oxyphil cells with an indistinct granulation occur in small numbers round the margins of the circumscribed tubercles and within the smaller and irregular tubercular areas. In the interalveolar tissue both the finely and the coarsely granular types are present, but are not numerous. Not many leucocytes are found within the glandular alveoli. Neither giant cells nor concentric aggregations of cells of epithelioid type are found. There is no evidence of proliferation of the cells of the glandular epithelium. There is considerable new formation both of connective tissue corpuscles and of blood capillaries.

Distribution of Bacilli.—Tubercle bacilli are present in enormous numbers. They form a dense zone, visible with a low power, throughout the periphery of the older, circumscribed tubercles, and are present, though in less abundance, in their central portions. In the smaller and irregular tubercular areas also they form dense aggregations visible with a low power. They are present in large numbers between and within the alveoli and within the alveolar epithelial cells. Large numbers of bacilli are often present within the same cell.

2. The Tissue remote from the Nipple.

General Histological Characters.—Pieces selected from two regions exhibit lesions of the same type, but more advanced in the one case than in the other; the tissues are highly vascular, and there is a good deal of hæmorrhagic exudation. The patches of tubercular infiltration and necrosis are abundant, and in the more advanced specimens have completely obliterated large areas of glandular alveoli. The tubercular areas are irregular in outline and not circumscribed; in the less advanced lesions it is evident that the process is spreading by way of the interstitial tissue. There are neither calcareous deposits nor giant cells in the tubercular areas.

Fibrin.—More fibrin, both old and recent, is demonstrable than in the sections described above.

Minute Characters of Cells.—Plasma cells are abundant, and similarly situated to those found near the nipple. The distribution and characters of oxyphil cells and of the fixed tissue cells are also similar.

Distribution of Bacilli.—Bacilli are even more numerous than near the nipple, and have a similar distribution. None are found within intact blood vessels.

Right Hind.

1. Advanced Lesions.

In one portion examined the lesions are similar in type to those found near the nipple of the left fore. There are both circumscribed and diffuse tubercular areas. The circumscribed tubercles are larger than in the left fore, and contain more abundant calcareous deposit; they also contain a few giant cells. In all other respects the lesions are similar, both histologically and bacteriologically, to those found near the nipple of the left fore.

2. Early Lesions.

Another portion examined exhibits an early stage of tubercular infection.

General Histological Characters.—Under a low power view the only pathological features observable are an increase of the interstitial tissue and a few giant cells near the margin of some of the sections.

Fibrin.—There is not a trace of fibrin, either where bacilli are present or elsewhere.

Minute Characters of Cells.—Plasma cells, staining faintly, are found in moderate numbers in the interstitial tissue. Multinuclear leucocytes are present, but infrequent. Oxyphil cells, not multinuclear and definitely granular, are also rare on the whole, but are found in occasional small aggregations, in areas where there is no histological appearance suggestive of tubercular infection. Small lymphocytes are fairly numerous in the interstitial tissue. A few giant cells are found; they all occur within the interstitial tissue.

Distribution of Bacilli.—In the interstitial tissue bacilli are occasionally found; sometimes they are lying free and sometimes they are contained within giant cells. Within some of the glandular ducts are found groups of desquamated epithelial cells containing several tubercle bacilli. These are the only two situations in which tubercle bacilli are found.

Characters of Bacilli in the two Infected Quarters.

The bacilli vary in length from $1\cdot5$ to 4μ, and average about $2\cdot5\mu$. About one-third are curved; very few are beaded.

Peculiarities of this Udder.

This udder is remarkable from the fact that although, clinically, running a very chronic course, it contains bacilli in enormous numbers. The distribution of bacilli is also unusual. Definitely circumscribed caseo-calcareous tubercles are generally poor in bacilli; in this case bacilli occur in enormous numbers in these situations, and form a well-marked peripheral zone.

The amount of tissue destruction is small relatively to the duration of the infection and the enormous numbers of bacilli present. In most acute udders, where the experiments were fatally terminated much sooner, there is more tissue destruction. Fibrin, though present in some situations where bacilli are numerous, is, in these localities, generally associated with capillary hæmorrhages; no fibrin is present in the areas of early infiltration where bacilli are scanty. In the areas of early infiltration many of the bacilli present are enclosed within giant cells; bacilli are also found lying free in these situations without any histological evidence of tissue reaction.

Summary.

A very slowly progressive tuberculosis, in which the amount of tissue destruction is small relatively to the enormous number of bacilli present.

VIRUS.—B II.

Animal Inoculated.—Cow 4.

Cow 4 received an intramammary inoculation, into the left fore and right hind quarters, of an emulsion of the original material. The animal was killed 34 days afterwards.

In the non-inoculated quarters there is marked œdematous infiltration of the interlobular and interalveolar tissue with proliferation of the connective tissue cells and aggregations of lymphocytes and plasma cells. The capillaries are numerous and distended with red corpuscles, and there are a few small patches of hæmorrhagic exudation. In some places the inflammatory process has extended beyond the interstitial tissue, and caused partial or complete obliteration of a single alveolus or a small group of alveoli. The gland cells are swollen and contain large fat vacuoles. Bacilli occur in considerable numbers in the interstitial tissue and in the inflammatory foci, which show commencing obliteration of the glandular alveoli. A few are seen lying free in the gland tubes or amongst the cells of the alveolar lining; in these cases the glandular epithelium is generally observed to be falling away and is in process of invasion by the cells of the interstitial tissue, amongst which the bacilli are present in much larger numbers. It is noticeable that many of the tubercle bacilli are intracellular. The cells which contain them are in some cases evidently of an epithelial type, but in many instances it is impossible to give a definite opinion as to their character. There are many small deposits of fibrin, which are in close association with the bacilli.

The inoculated quarters are identical in microscopic characters with those of Cow 18 (B I.). The transitional areas between the extensively diseased and the relatively normal portions of the udder, and the condition of the tissue surrounding the milk ducts and sinuses, are also similar to those of Cow 18.

Bacilli are very numerous, and are morphologically identical with those found in Cow 18.

Summary.

Acute progressive infection, with rapid tissue destruction, œdema of the surrounding parts, and great multiplication of bacilli.

VIRUS.—B II.

Animal Inoculated.—Cow 500.

Cow 500 was inoculated in the left fore and right hind quarters, with the same dose of the same material as Cow 4. The animal was killed, when in a moribund condition, 32 days afterwards.

In the non-inoculated quarters there is considerable increase of the interstitial fibrous tissue, and a few capillary hæmorrhages have been noted. No tubercle bacilli have been found.

The microscopic characteristics of the infected quarters of this animal's udder are hæmorrhage, œdema, and diffuse necrosis. In the older lesions there is a notable absence of conservative tendencies. The degenerated areas are not circumscribed by definite boundaries of fibrous tissue; there is no indication of calcification, and though patches of infiltration with small round cells are still to be seen, there is no evidence of vigorous reaction on the part of the fixed connective tissue elements; the entire tissue has given way to necrotic changes. In order to study earlier stages in the process, I have prepared sections which include each side of what appeared macroscopically the line of demarcation between healthy and diseased tissue. Here microscopic examinations shows that the normal fibrous tissue septa between the gland lobules are still intact. On the one side of the broader of these septa we have unchanged glandular tissue, on the other infected tissue. The lobules on the latter side show different stages of an intense inflammatory reaction; they are packed with lymphocytes and polymorphonuclear leucocytes, and show rapid proliferation of the fibrous tissue the consequence being extensive and rapid obliteration

of the glandular elements. Tubercle bacilli are very numerous throughout the infected quarters. The great majority of them are straight; many are beaded definitely throughout their length; still more are stained irregularly rather than distinctly beaded; the rest are stained more or less homogeneously. Many are somewhat thick, and some of these appear to be slightly narrower in the middle. The average length is a little over 2μ. Some bacilli are particularly short and thick, measuring not more than $1\cdot5\mu$. Curved forms also occur.

SUMMARY.

Acute infection, with copious exudation and diffuse necrosis rather than circumscribed caseation. Rapid multiplication of bacilli.

VIRUS.—B III.

Animal Inoculated.—Cow 68.

Cow 68 received an intramammary inoculation into the left fore and right hind quarters of an emulsion of the original material. The dose inoculated into each quarter was estimated to contain 14,600,000 bacilli. The animal was killed forty-eight days after inoculation.

General Histological Characters.—In the non-inoculated quarters the only pathological changes observable, except in immediate proximity to the inoculated quarters, are small groups of pus corpuscles in a few of the alveoli, and some interstitial inflammation.

In the inoculated quarters, although the tissue was calcareous, the calcareous deposits were individually small, and sections could be prepared, though with difficulty, without previous decalcification. It was thus possible to study the details of this advanced type of tubercular lesion without the damage to finer details which always results from decalcification. Many sections are largely occupied by necrotic areas in which the tissue cells have disappeared, and isolated calcareous foci are conspicuous. A striking feature about many of these necrotic areas is the presence within them of numerous blood-vessels and capillaries filled with unaltered red blood corpuscles. The necrotic patches are not bounded by any definite fibrous tissue zone, but are diffuse, irregular in outline, and confluent. In areas which have not advanced to necrosis the lesions are also characterised by diffuse tubercular infiltration, without the formation of circumscribed tubercles, and enclose here and there islands of almost unaltered glandular tissue. In the earliest areas of commencing tubercular infiltration there is considerable interstitial œdema. Near the line of contact between the non-inoculated and inoculated quarters there are, in the former, small tubercular areas which are almost completely isolated, and contain numerous giant cells, differing in these two respects from the general type of lesion found in the inoculated quarters.

Fibrin Formation.—In the necrotic areas dense patches and strands of material which take a fibrin stain are found. There are also, in the earlier lesions, some patches of delicate fibrin fibrils; but these are not very frequent.

Minute Characters of Cells.—At the margin of necrotic areas are found, passing from within outwards, the following types of cell elements:—(1) Small round cell nuclei, not surrounded by any protoplasm, which stain deeply by Pappenheim's method and by methylene blue; (2) in addition to these, much larger cell nuclei, oval, irregular, or spindle-shaped in outline, staining pale green by Pappenheim's method and pale blue with methylene blue, surrounded by indistinct, unstained protoplasm; (3) in addition to both these types, plasma cells with a large amount of protoplasm staining red by Pappenheim's method and blue with methylene blue; in associa-with these are a number of smaller cells with rounded nuclei and surrounded with a very thin margin of protoplasm which takes a red pyronin stain; these would probably be called "daughter" plasma cells by Pappenheim; (4) in addition to these three types, connective tissue corpuscles, the protoplasm of some of which takes a red pyronin stain. Leucocytes of the polymorphonuclear type are not numerous, except in proximity to blood-vessels, where they are occasionally abundant.

In areas which have not begun to necrose, but where no traces of normal glandular tissue are left, we have a confused mass of cells of the "epithelioid" and "small round" types intermingled. On minute examination, most of these cells present the characters of the cells specified under the above four headings. Polymorphonuclear leucocytes occur, but not very abundantly. In eosin and methylene blue specimens their protoplasm stains pink, and shows a fine granulation; it is in marked contrast to the blue-stained protoplasm of most of the surrounding cells. Oxyphil leucocytes with a spherical nucleus are rarely met with.

In earlier areas, showing infiltration of gland tissue, are to be found large plasma cells, "daughter" plasma cells, small lymphocytes with no demonstrable protoplasm, young connective tissue corpuscles, and a variable number of polymorphonuclear leucocytes. In many sections this last type of cell is very rare; in others it is abundant. Where abundant, large numbers of pus corpuscles are found in neighbouring glandular alveoli, and it is difficult to decide whether the leucocytes in the interstitial tissue are directly associated with the tubercular process.

Giant cells are rare, except in the situation already referred to.

Distribution and Characters of Tubercle Bacilli.—Tubercle bacilli are, on the whole, scanty. In the necrotic areas a few are generally to be found, and it is a striking feature that bacilli occur within most of the calcareous nodules. In these situations they are either scattered singly or in groups of about half a dozen; although embedded in calcareous material, they stain well, and do not, as a rule, exhibit an irregular or degenerate appearance. I have frequently noticed that bacilli are less infrequent in these calcareous patches than in the surrounding necrotic tissue, and that in the latter situation they often exhibit degenerate forms. In lesions where the cells have not begun to necrose bacilli are again infrequent, and in areas of early infiltration hardly any are to be found. In the exceptional situation recorded, where the tubercles are more or less isolated and giant cells are numerous, two or three bacilli are to be found in most of the giant cells, and a few single bacilli, some of them intracellular, occur amongst the neighbouring cells.

The bacilli varied in length from $1\cdot5$ to $4\cdot5\mu$, and occasionally longer forms were noted. The average length was about $2\cdot5\,\mu$. About one-third were curved; nearly three-fourths were either regularly beaded or irregularly stained, globular swellings being common.

SUMMARY.

Progressive tuberculosis of the caseous type, with a tendency to calcification. Evidence of vigorous, though unsuccessful, resistance on the part of the tissues. Bacilli not very abundant.

VIRUS.—B IV.

Animal Inoculated.—Cow 172.

Cow 172 received an intramammary inoculation into the left fore and right hind quarters with an emulsion of the tissue of Calf 138. The dose inoculated into each quarter was estimated to contain 14,670,000 bacilli. The animal was killed 392 days afterwards.

Specimens from each of the four quarters have been examined. They all show lesions and are very much alike in microscopic appearance. The lesions found in the right hind, one of the inoculated quarters, are, however, somewhat more advanced than the lesions found in the non-inoculated quarters. In all the sections, circumscribed tubercles are present. Some of these are in an early and some in an advanced stage of caseation, but they are all of the same type. They are bounded by a broad layer of fibroblasts and contain numerous giant cells. The interstitial tissue between the alveoli and between the glandular lobules is very much increased; some of the caseous foci are found in this situation, and it appears probable that the infection is spreading by way of the interstitial tissue, with the result that islands of parenchymatous tissue are cut off from time to time and become involved in the tubercular foci. Plasma cells are very abundant in the interstitial tissue and at the periphery of the tubercles. There is no general infiltration with oxyphil leucocytes but groups of these cells are found in many of the caseating foci. Coarsely granular oxyphils and mast cells are rather numerous in the interstitial tissue. No fibrin is demonstrable in any of the specimens. Tubercle bacilli are present, generally in the caseous foci or within giant cells, but are not numerous.

Summary.

Slowly progressive tuberculosis of the chronic type.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 1. C.M.

Animal Inoculated.—Cow 3.

Cow 3 received an intramammary inoculation, into the left fore and right hind quarters, of a potato culture of this virus which had been isolated from a guinea-pig; the cow was killed 63 days afterwards.

One of the non-inoculated quarters, the left hind, is histologically normal and contains no tubercle bacilli.

The other non-inoculated quarter, the right fore, shows several patches of interstitial mastitis, and in one region groups of small tubercles have been found. These are centrally caseous and are surrounded by a broad cellular zone which is to a large extent fibrous. In several of these tubercles bacilli are present, but only in small numbers.

In both the inoculated quarters the greater part of the mammary tissue has been replaced by typically tubercular foci. These foci are as a rule circumscribed, being surrounded by a dense environment of fibrous tissue, and present according to their size a varying amount of degenerative change. In the largest of them there is a small calcareous nodule in the centre, not of sufficient size to require decalcification of the tissue; external to that a zone of caseous material; and between the latter and the outer border of fibrous tissue a belt of endothelial cells, leucocytes, and lymphocytes, with an occasional giant cell, the last measuring on the average about 35μ in its longest diameter. In somewhat smaller nodules the central portion is simply necrotic, without any calcification; and in still more recent nodules there is no caseation, the central portion being occupied by large protoplasmic, endothelial or "epithelioid" cells, and sometimes containing well formed giant cells. In many sections transitional changes may be observed between the tubercular areas where there is no recognisable mammary tissue left and portions of intact glandular tissue. The tubercular process appears to have spread by way of the interstitial tissue. The earliest change is an increase and infiltration of the connective tissue elements; then follows a compression, and finally a complete obliteration of the glandular elements. The large protoplasmic cells, giant cells, and other constitutents of the tubercle, appear to arise at a time subsequent to the disappearance of the secretory cells. Plasma cells are very abundant at the periphery of the tubercles and in the interstitial tissue. Many of the glandular alveoli are filled with oxyphil leucocytes. No fibrin is demonstrable.

Tubercle bacilli are present in most of the tubercles, and in some they are numerous, from 40 to 50 being found in one microscopic field. They vary in length from 2 to 5μ, averaging over 3μ. A large number of them are curved and regularly beaded.

Summary

A chronic, slowly progressive tuberculosis.

VIRUS.—H 7. C.M.

Animal Inoculated.—Cow 73.

Cow 73 received an intramammary inoculation into the left fore and right hind quarters with an emulsion of the original material. The animal was killed 178 days afterwards.

Left Fore Quarter.—The interstitial tissue between the lobules is dense and contains a large proportion of elastic fibres. The groups of glandular alveoli are compressed, and the lumen of some of the alveoli is obliterated. The individual alveoli are separated from each other by dense accumulations of cells, some of which have a small round nucleus surrounded with little or no protoplasm, and are identical in appearance with small lymphocytes; a great number of the cells found in this situation are young connective tissue corpuscles, and a few are cells which react to Pappenheim's stain for plasma cells. There is no histological appearance which is typical of a tubercular process. The general characters of the tissue are those of a subsiding mastitis.

I have searched several sections which have been stained for tubercle bacilli, but have not found any.

The Left Hind and the Right Fore and Hind Quarters are all histologically normal. Sections from each of these three quarters have been stained for tubercle bacilli, but no bacilli have been found.

Summary.

A subsiding mastitis; no evidence of tuberculosis.

VIRUS.—H 10. B.S.

Animal Inoculated.—Heifer 249.

Heifer 249, aged about 2 years, was inoculated subcutaneously with 50 mg. of a culture of this virus. The animal was killed, when very ill, 54 days afterwards.

The interstitial tissue in the specimens examined is infiltrated with small lymphocytes and plasma cells, but contains only a few oxyphil leucocytes. Two or three foci have been found which are slightly caseous and contain giant cells. Tubercle bacilli are present in these areas but are rare, only two having been found in the whole of one section.

Summary.

Very slight tuberculosis.

VIRUS.—H 10. B.S.

Animal Inoculated.—Cow 295.

Cow 295 received an intramammary inoculation, into each of two quarters, with an emulsion of tissue from Calf 199. The dose inoculated into each quarter of the udder was estimated to contain about 2,000,000 bacilli. The cow died suddenly after parturition, 179 days after being inoculated.

In each of the inoculated quarters a few isolated tubercles, in an advanced stage of caseation, have been found. Tubercle bacilli are present in the lesions but are very scanty.

Summary.

A few isolated, caseous tubercles, in which bacilli are rare. As the virus inoculated is of high virulence, the amount of disease produced is remarkably small.

VIRUS.—H 14. F.S.

Animal Inoculated.—Cow 75.

Cow 75 received an intramammary inoculation, into the left fore and right hind quarters, of an emulsion of tissue from Calf 125. The dose inoculated into each quarter was estimated to contain 19,000,000 bacilli. Cow 75 was killed when very ill, 34 days afterwards.

General Histological Characters.—In the non-inoculated quarters there is a little œdematous infiltration, but no other noticeable pathological change. The fibrous tissue septa are moderately dense.

In the inoculated quarters there is hardly any evidence of calcareous degeneration. The few calcareous foci found are small; the starting-point of some of them appears to have been a calcareous concretion in a glandular duct or alveolus. Many sections exhibit extensive necrotic change, with obliteration of the tissue cells, but without caseous softening or breaking down. These necrotic areas show no definite fibrous tissue demarcation; they are diffuse, irregular, and generally confluent; they contain numerous small blood capillaries filled with red corpuscles. In the less advanced areas of infection and in the transitional regions necrotic patches are less numerous and smaller, whilst patches of the "epithelioid" type of tubercle are more prominent.

Fibrin Formation.—Fibrinous exudations are well demonstrated by Kochel's method, and in eosin and methylene blue specimens, in all the infected tissue. None are present in the tissue of the non-inoculated quarters. In the areas of less advanced infection, where the exudation is less dense and the fibrin fibrils are more delicate, tubercle bacilli are always to be found associated with the fibrin, even when absent from adjacent areas. But in some places tubercle bacilli are numerous, although there is not the slightest trace of fibrin formation; in these cases I note that many of the bacilli are intracellular.

Minute Characters of Cells.—The cellular elements resemble those found in other cases of acute infection, but, as compared with experiments which have run a longer course, contain more cells undergoing mitotic division. They also contain, on the whole, more polymorphonuclear leucocytes; these seem to be most numerous in the stage just preceding necrosis; they are also found in the tissue near to glandular alveoli which contain large numbers of pus corpuscles. A few giant cells are to be found.

Distribution of Tubercle Bacilli.—Bacilli occur in fairly large numbers practically throughout the infected quarters. In a few sections they form groups visible with a low power of the microscope. They are abundant in the tissue near the nipples of the inoculated quarters. Compared, however, with the three udders, acutely infected with bovine bacilli, on which I have previously reported (Cows 4, 18, and 500), the number of bacilli in the udder of Cow 75 is considerably smaller. Bacilli are, however, much more numerous than in the udder of Cow 68, which was inoculated with bovine bacilli. With regard to the relative numbers of bacilli in the different phases of the tubercular lesions found in this udder, the number appears to be greatest at or about the point of commencing necrosis. Before necrosis has commenced there is a fair sprinkling of bacilli amongst the tubercular tissue cells. A very considerable number of these bacilli are contained within the cells; it is difficult to define the exact types of cells in which the bacilli occur, but most of them are certainly not leucocytes; they are cells of a larger type, and have an oval or slightly elongated or rounded nucleus to which the bacillus is often closely applied. I have only occasionally, in these sections, found a bacillus within a typical leucocyte. Sometimes bacilli are found, either free or intracellular, within the lumen of ducts or alveoli. I have searched several groups of cells, consisting of detached epithelial cells and pus corpuscles, lying free in these situations, and containing intracellular bacilli. The cells containing the bacilli are much more frequently of the former than of the latter type. In the areas of just commencing tubercular infiltration bacilli are very rare; in this respect, again, this udder contrasts with the acute cases infected with bovine material above alluded to.

Characters of Bacilli.—The bacilli vary in length from 1·5 to 5 μ and average about 3 μ. About one-third are curved and three-fourths are beaded or irregularly stained. The beading is often well marked.

Summary.

Acute, progressive tuberculosis; compared with udder infected with bovine bacilli, the morbid process is perhaps slightly less acute than in the cases of Cows 4, 18, and 500, and rather more acute than in the case of Cow 68.

VIRUS.—H 19. S.W.

Animal Inoculated.—Heifer 233.

Heifer 233 was inoculated subcutaneously with 53,440,000 bacilli contained in an emulsion of the tissues of Calf 271 and was killed 181 days subsequently. Fifty-four days after inoculation, the animal gave birth to a healthy calf.

Right Fore.—Normal histologically. No tubercle bacilli present.

Right Hind.—There is extensive pathological change. In the areas which appeared to the naked eye to be most severely affected, the greater number of the mammary alveoli are oblitered, being replaced by a cellular infiltration which exhibits many patches of commencing necrosis, and contains numerous structures resembling giant cells; between those alveoli which retain their normal appearance, the interstitial tissue is much thickened and infiltrated. In the areas which, to the naked eye, appeared less severely affected, the microscopic changes are the same in type, but less advanced, the amount of infiltration and necrosis being less, and the number of intact alveoli being larger.

Both in the more advanced and in the earlier lesions tubercle bacilli are present, but are very rare; out of six sections examined two contained, each, three or four bacilli, the rest none. The bacilli were found in breaking down material and in cell-fusions, not in leucocytes.

Oxyphil multinuclear leucocytes are abundant in the alveoli, but not nearly so numerous as small lymphocytes in the interstitial tissue. The predominant feature of this tissue is an infiltration with small lymphocytes; associated with these are a relatively small number of plasma cells. Whilst some of the giant cells may be typical new formations, the majority of these appearances are undoubtedly nothing more than breaking down mammary alveoli. In van Gieson specimens the fibrous tissue does not appear to be increased in amount. Where a tract of fibrous tissue is observed at the periphery of a semi-necrotic area, it can be generally traced in continuity with the normal interlobular connective tissue, and is not suggestive of the fibrous tissue demarcation of a typical tubercle.

Left Fore.—The normal mammary structure is nowhere obliterated, but the tissue is characterised by an invasion of oxyphil multinuclear leucocytes. Many of the ducts and alveoli are packed with these cells; they are present in very large numbers in many parts of the interalveolar interstitial tissue, and can be traced passing, from this situation, in between the mammary epithelial cells. In several parts of the interalveolar interstitial tissue there are also considerable aggregations of small lymphocytes and of plasma cells.

There are no tubercle bacilli, nor is there any sign of caseation or of giant cell formation.

Left Hind.—Normal histologically. No tubercle bacilli present.

Summary.

Slight tuberculosis, probably subsequent to a non-tubercular mastitis following parturition. The tubercular lesions here found offer an interesting resemblance, histologically and in the rarity of bacilli, to some cases of human mammary tuberculosis.

VIRUS.—H. 19. S.W.

Animal Inoculated.—Heifer 239.

Heifer 239 received a subcutaneous inoculation with an emulsion of tissue obtained from Calf 159. The dose was estimated to contain 1,100,000 bacilli. The heifer was killed 177 days subsequently.

Specimens from two portions of this udder have been examined. Both show minute tubercular foci characterised by commencing caseation. These foci generally contain partially disintegrated alveoli, which somewhat resemble giant cells; occasionally cell-fusions or aggregations of nuclei are found which cannot be explained in this way, but are undoubtedly new formations. The interalveolar and interlobular tissue is densely infiltrated with small lymphocytes, and also contains some plasma cells and a few oxyphil leucocytes. The tubercles are surrounded by dense masses of small lymphocytes, and sometimes contain small groups of leucocytes. No fibrin is present. Tubercle bacilli are present in some of the lesions, but are very rare. Only two have been found in five sections.

Summary.

Very slight tuberculosis.

VIRUS.—H 19. S.W.

Animal Inoculated —Calf 597.

Calf 597 was inoculated subcutaneously with 50 mg. of a culture isolated from Heifer 239, and was killed, when moribund, 45 days afterwards.

Two portions of the udder have been examined. Both contain many areas of early caseation which are surrounded by an infiltration of small lymphocytes and contain some leucocytes, but are irregular in outline and exhibit no fibrous tissue barrier. It is noteworthy that many of these areas do not involve the mammary alveoli, but are in immediate proximity to the larger milk ducts. Tubercle bacilli are present in all the caseous foci; two or three are generally found in every microscopic field.

Summary.

Early, progressive tuberculosis.

VIRUS.—H. 28. C.L.

Animal Inoculated.—Cow 143.

Cow 143 received an intramammary inoculation into the the left fore and right hind quarters of an emulsion of tissues from Calf 515. The dose inoculated into each quarter was estimated to contain 20,000,000 bacilli. The animal was killed, when in a moribund condition, 38 days afterwards.

The two inoculated quarters were found to be enormously enlarged and were diseased throughout. Microscopically they show advanced, diffuse caseation, with no demarcation into tubercles. Bacilli are present throughout the tissue, but not in large numbers. A few small patches of fibrin have been found.

SUMMARY.

Acute progressive tuberculosis with rapid tissue destruction, but a relatively slow increase in the numbers of bacilli.

VIRUS.—H 29. M.F.

Animal Inoculated.—Heifer 251.

Heifer 251 received a subcutaneous inoculation with a tissue emulsion from Calf 479. The dose was estimated to contain nearly 3,000,000,000 bacilli. The animal remained in apparently good health and was killed after the lapse of 138 days. Nine days before the termination of the experiment the heifer gave birth to a calf.

Two portions of the udder have been examined. Both contain many patches of early caseation, in which the gland epithelium has been partially obliterated. Both in these areas and within the alveoli of the surrounding tissue oxyphil leucocytes are very numerous. Tubercle bacilli are present in scanty numbers in the foci which have the definite appearance of tubercles. They are also found in some of the patches of leucocytic infiltration which cannot, histologically, be recognised as tubercles; but they are nowhere numerous.

SUMMARY.

An early and apparently progressive tuberculosis.

VIRUS.—H 29. M.F.

Animal Inoculated.—Calf 477.

Calf 477 was killed, when moribund, 34 days after a subcutaneous inoculation with an emulsion of the organs of guinea-pigs which had been inoculated with the original material. The dose was estimated to contain about 3,000,000,000 bacilli.

Two portions of the udder have been examined. They both contain fairly large caseous areas. These are not circumscribed by fibrous tissue, but appear to be spreading. They contain fairly large numbers of bacilli. A few bacilli have also been found in parts of the interstitial tissue where there is no histological evidence of tuberculosis. No fibrin is demonstrable.

SUMMARY.

Progressive tuberculosis.

II. EXPERIMENTAL TUBERCULOSIS OF THE LUNGS.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Heifer 8.

Heifer 8 received an intravenous inoculation of bacilli contained in an emulsion of the organs of guinea-pigs which had been inoculated with the original material. The animal was killed, when very ill, 25 days afterwards.

General Histological Characters.—Sections taken from the portions of the lungs which appeared to be completely solidified show entire obliteration of the normal lung structure. The tissue is completely consolidated and highly vascular; it is necrotic in some places; but there is no general feature as seen under a low power which is peculiar to tuberculosis; the general appearance is that of a pneumonic consolidation of the lobar type. In other portions of the lung tissue the patches of consolidation are small and relatively isolated, though tending to become confluent; they are highly vascular, and are often adjacent to patches of lung alveoli which are filled with a catarrhal exudate. In the least affected portions of the lungs the alveoli are patent, but the alveolar walls are thickened and the blood capillaries dilated.

Fibrin.—Fibrin is present, but appears to have partly cleared up. More is found in the small than in the large patches of consolidation.

Minute Characters of Cells.—Plasma cells are present, particularly around the blood-vessels, but do not stain well. In eosin and methylene blue specimens eosinophil cells of various types are numerous, and are distributed irregularly throughout the tissue. Some of the oxyphil leucocytes are distinctly granular, but there are a much larger number of ordinary multinuclear leucocytes in which granulation is either absent or indistinct. Small lymphocytes are also found. With regard to other types of cells present, in the small foci of consolidation the fixed tissue cells are obscured by the infiltration of leucocytes and of large numbers of red blood corpuscles; the epithelial cells have desquamated or disappeared, and the proliferation of connective tissue or other elements is not extensive. In the larger patches of consolidation the leucocytic infiltration is still the predominant feature, but there is more evidence of the irregular proliferation of cells of connective tissue and endothelial type, without the formation of any definite structure.

Distribution and Characters of Bacilli.—Tubercle bacilli are very numerous and are dispersed throughout the tissue, sometimes in large clusters. They are frequent in hæmorrhagic areas but are not noticed within any intact blood-vessel. There is no evidence of a mixed infection. The bacilli vary in length from 1 to 3·5μ, the average being about 2μ. Most of them are straight.

SUMMARY.

The lung is consolidated. Though this condition has obviously been brought about by the presence of enormous numbers of tubercle bacilli, the lesions have not the patchy distribution into centrally caseous areas which is typical of tubercular pneumonia.

VIRUS.—B 1.

Animal Inoculated.—Heifer 10.

Heifer 10 received an intravenous inoculation of a culture isolated from G.P. 14, which had been inoculated with the original material. The heifer was killed 37 days afterwards.

General Histological Characters.—In sections examined from three portions of the lungs the greater part of the normal lung structure is obliterated, and replaced by irregular areas of consolidated tissue, which are partially confluent and often show patches of caseation. In van Gieson specimens the amount of the fibrous tissue elements is seen to be increased in these areas, and to have an irregular distribution. The rest of the lung tissue is highly oedematous, and the majority of the alveoli are filled with a coagulated exudate. The pleura covering the lung is thickened, highly vascular, and contains isolated nodules which have the characteristics of circumscribed tubercles.

Fibrin.—Deposits of very early fibrin and also of older fibrin are found in abundance throughout the tissue.

Minute Characters of Cells.—Plasma cells are present in irregular patches in large numbers; many of them are in process of transition into fibroblasts. Connective tissue corpuscles are abundant, and appear to be in active growth. The cells of epithelial type are not arranged in any definite structure; their protoplasm shows signs of disintegration, and the groups in which they occur are invaded by leucocytes and connective tissue cells. Multinuclear leucocytes of the finely granular type are very numerous, both in the areas of tissue consolidation and elsewhere; some of the bronchi are packed with them. Coarsely granular cells are not found. Small lymphocytes, with a large spherical, deeply and homogeneously staining nucleus, are not numerous.

Distribution of Bacilli.—Tubercle bacilli are swarming throughout the lung, often forming groups visible with a low power of the microscope. None have been found within the lumen of intact blood-vessels.

Characters of Bacilli.—The bacilli vary in length from 1·5 to about 4μ, and average about 2.75μ. Curved and beaded or irregularly stained forms are numerous.

SUMMARY.

A typical miliary tuberculosis which is becoming confluent. Evidence of vigorous, though unsuccessful, tissue resistance. Great multiplication of bacilli.

VIRUS.—B I.

Animal Inoculated.—Heifer 26.

Heifer 26 was inoculated intravenously with an emulsion of the udder of Cow 40. This udder contained very few bacilli. The heifer was killed 115 days after inoculation.

General Histological Characters.—The lungs contain definitely circumscribed and completely isolated tubercular nodules. The larger of these are bounded by a very dense fibrous tissue zone, contain a good deal of adult fibrous tissue throughout their substance, and are caseating in the centre. Except in their centres, the nodules are richly supplied with blood-vessels. On the pleural surface, adjoining one of the tubercular nodules, is a tufted outgrowth consisting, as seen under a low power, of a widely open and irregularly arranged meshwork of delicate

fibrils, amongst which are several blood-vessels and a scanty number of cells, the whole being bounded by a single layer of pleural epithelium. Apart from the tubercular nodules, the substance of the lung is normal.

Fibrin.—In the pleural outgrowths there is an entire absence of anything of a fibrinous character. In the tubercular nodules there is no satisfactory evidence of fibrin.

Minute Characters of Cells.—Plasma cells are well shown and abundant in the outer half of the tubercular nodules. Elastic fibres are not numerous within or surrounding the tubercles, and are not present in the pleural outgrowths. These outgrowths are completely external to the normal elastic tissue boundary of the lung. The cellular portions of the tubercles are packed with an enormous number of eosinophil cells. They occur in every field and in many areas amount to from one-twelfth to one-fifth of the total number of cells in focus; amongst the multinuclear forms there is every degree of transition from the smaller to the larger forms, and from those with a dark blue to those with a pale blue nucleus; a great many of these cells only differ from the ordinary polymorphonuclear leucocyte in the fact that their granules are more distinct, and larger than usual. Small lymphocytes are also present, but not numerous. Cells of epithelioid form are also found. The cells between which the oxyphil cells are packed are partly connective tissue cells, partly plasma cells, and partly cells about which, owing to their compression, it is impossible to say whether they are of endothelial or connective tissue type. Beneath the pleura, and in the pleural outgrowths are found cells with a round and rather lightly staining nucleus, small cell bodies, and fibrillar elongations; there are also a moderate number of oxyphil cells. In the normal parts of the lung tissue oxyphil leucocytes occur here and there in the alveolar walls, but not in the lymphatic tissues.

Distribution of Bacilli.—In the central portions of the nodules tubercle bacilli are found, but are very scanty. They are not found elsewhere.

Summary.

Chronic tuberculosis, characterised by the presence of isolated tubercles surrounded by a broad fibrous zone, scarcity of bacilli, and the absence of any indication that the lesions are still progressive.

VIRUS.—B I.

Animal Inoculated.—Heifer 30.

Heifer 30 was inoculated subcutaneously with an emulsion of the udder of Cow 40 and was killed 115 days subsequently.

General Histological Characters.—In the lung substance are isolated nodules, which are separated from the rest of the lung tissue by a dense fibrous tissue zone. The larger of these nodules are caseous in the centre. They all contain a large amount of fibrous tissue stroma. They contain a few giant cells, and are vascular except in the patches of commencing or advanced necrosis. The rest of the lung substance is normal.

Fibrin.—Some of the deposit at the margin of the larger nodules and in the vascular areas appears to be fibrinous.

Minute Characters of Cells.—Plasma cells are abundant in the nodules; many of them are passing into fibroblasts. Within the nodules there is very little elastic tissue; and elastic fibres are not numerous round the margins of the nodules. In the peripheral portions of the nodules there are present in enormous numbers cells which in eosin and methylene blue specimens are filled with rather coarse eosinophil granules. At least four varieties of these cells are present: (1) In small numbers, small cells, a little over 4μ diameter, with a spherical, deeply and uniformly stained nucleus and a narrow rim of protoplasm, which is thicker at one side than the other. (2) In very large numbers, cells with a polymorphous deep-blue nucleus, a fair amount of protoplasm, and an irregular or oval outline; these cells vary very considerably in size; many of them measure about 5μ by 8μ, some are much smaller, and a few are larger. (3) In considerable numbers, cells with an irregular pale-blue nucleus, a large amount of protoplasm, and an irregular, often somewhat oval, cell outline; many of these cells are rather larger than those last described; (4) Occasionally cells of about the same size as the last, but with a much larger nucleus, which is pale blue, regular, and of oval outline. (5) In some places several nuclei of the polymorphous type are massed together and surrounded by granules, without any evident demarcation between the protoplasm belonging to each nucleus. Lymphocytes, with a spherical deeply stained nucleus and no recognisable protoplasm, are present in moderate numbers; they are less numerous than the eosinophil cells. Patches of cells of epithelioid type are found in the centre of the smaller tubercles and near the periphery of the larger tubercles; they are partially disintegrating, and stain badly. The pale areas in which these groups of cells occur are best differentiated in eosin and methylene blue specimens.

Distribution of Bacilli.—Tubercle bacilli occur within the tubercular nodules, near their periphery. Even in these situations they are very rare; none have been found in other situations.

Summary.

Chronic tuberculosis, similar to that produced by the intravenous inoculation of the same material into Heifer 26.

VIRUS.—B I.

Animal Inoculated.—Cow 40

Cow 40 received an intramammary inoculation with a culture isolated from G.P. 14, which had been inoculated with original material. Cow 40 was killed ninety-two days after inoculation

General Histological Characters.—The sections examined contain isolated tubercles of irregular outline. Most of the tubercles are caseous and contain a calcareous nodule in the centre. A good deal of fibrous tissue, staining red in van Gieson specimens, is found within them, but they are not circumscribed by a definite fibrous tissue zone. Except in the areas of calcification or complete caseation, the tubercles are vascular; numerous capillaries, filled with red blood corpuscles, extend inwards close up to the caseous zone. The rest of the lung tissue contains a few patches of catarrhal exudation, but is otherwise normal.

Fibrin.—There is no evidence of fibrinous deposit.

Minute Characters of Cells.—The outer portions of the tubercles contain numerous plasma cells; cells of this type also surround some of the bronchioles. Multinuclear leucocytes of the finely granular oxyphil type are abundant in the cellular portions of the tubercles; they only occur in small and apparently normal numbers in the rest of the lung tissue. In addition to a large number of fibrous tissue corpuscles, the tubercles also contain cells with large rounded nuclei, and there are some giant cells with nuclei of this type; the staining reactions of these tubercles are not sufficiently good to

render more minute differentiation possible. In the interstitial tissue of the lung septa are a considerable number of large cells filled with coarse granules, which stain a reddish purple in eosin and methylene blue specimens. These cells are also present, but in smaller numbers, in the alveolar walls. They do not occur in the tubercular areas.

Distribution of Bacilli.—Tubercle bacilli occur in the tubercular nodules, but are extremely rare. They are not found elsewhere.

SUMMARY.

Isolated caseo-calcareous tubercles, poor in bacilli and surrounded by a dense fibrous zone.

VIRUS.—B I.

Animal Inoculated.—Calf 48.

Calf 48 was inoculated subcutaneously with an emulsion of the spleen of Monkey 18. This strain had previously passed through Monkeys 2 and 20. Calf 48 was killed 185 days after inoculation.

General Histological Characters.—Sections have been prepared from portions of lung which show microscopic tubercles. The tubercles examined are about one-sixth of an inch in diameter and are situated in the midst of normal lung tissue. They are centrally caseous, with small calcareous foci ; external to the caseous zone are numerous large giant cells ; there is a well formed fibrous tissue layer marking off the tubercles from the rest of the lung tissue. The tubercles are very vascular except in the actually necrotic parts.

Fibrin.—No fibrin is present.

Minute Characters of Cells.—Plasma cells are found round the margin of the tubercles and surrounding adjacent bronchioles. In eosin and methylene blue specimens granular leucocytes, a large majority of them multinuclear, are very abundant round the margin of the nodules. In characters and situation these cells are almost identical with those found in Heifer 26. Every leucocyte present is distinctly granular, and the granules, though varying a little in size in different cells, are on the whole fairly large ; the average size of the granules in the multinuclear leucocytes with a deep blue nucleus is, perhaps, a little less than that of the very large eosinophils with a pale nucleus, or than the purplish granules of the mast cells. Cells of this last character are found in fair numbers in the interlobular connective tissue of the lung and occasionally at the periphery of tubercular nodules. Multinuclear and definitely granular leucocytes of medium size are present in small numbers in the normal portions of the lung tissue. In the small lymphatic patches in the normal lung tissue no oxyphil cells are noted except an occasional one in immediate proximity either to a bronchiole or an alveolus. Small lymphocytes are present in the tubercles, but are not at all numerous. Epithelioid cells enter largely into the structure of these tubercles, though they are infiltrated and partly overlaid by cells of other types ; they are best seen near the margins of caseation. Many giant cells are seen in process of formation and exhibit a close relationship to the epithelioid cells ; they occur where these cells occur, and have the same oval type of nucleus ; transitional stages can be observed between aggregations of partially discrete epithelioid cells and giant cells. There are found in these tubercles many newly-formed blood capillaries partly filled with red corpuscles, and also empty capillary channels ; some of the latter are cut in longitudinal sections. The relationship of the endothelium of these channels to the epithelioid cells is so close as to suggest a common origin.

Distribution of Bacilli.—Tubercle bacilli are very rare and are confined to the tubercular nodules.

SUMMARY.

Isolated, chronic, apparently non-progressive tubercles, poor in bacilli.

VIRUS.—B I.

Animal Inoculated.—Calf 52.

Calf 52 was inoculated subcutaneously with the spleen of Monkey 2 (*i.e.*, before the strain had passed through Monkeys 20 and 18) and was killed 32 days subsequently.

General Histological Characters.—Throughout the specimen are small areas of consolidated tissue, irregular in outline and without fibrous tissue demarcation ; many of them are partially necrotic, but there is no differentiation into typically tubercular zones. The lesions vary considerably in size and frequency ; in some places they appear to be spreading and becoming confluent and there is infiltration of the adjacent interalveolar tissue.

Fibrin.—Fibrin is present in considerable quantity in the consolidated areas.

Minute Characters of Cells.—With Pappenheim's stain a small number of cells which take a faint red protoplasmic tinge are found within and in the neighbourhood of the consolidated areas. Cells which take the nuclear stain of small lymphocytes are numerous throughout the tissue. Polymorphonuclear and mononuclear leucocytes are present in large numbers, both in the consolidated areas and in the rest of the lung tissue. Nearly all the polymorphonuclear leucocytes are of the ordinary type, being either not distinctly granular or only finely granular. In the blood-vessels the eosinophil cells do not appear to be much in excess of the normal. Apart from desquamated alveolar cells, cells of epithelioid type are rare. There are a good many broken down red blood corpuscles in the consolidated areas. No giant cells have been noticed.

Distribution of Bacilli.—Tubercle bacilli are present in moderate numbers in the consolidated areas, chiefly in the necrotic parts ; not many of them are intracellular. None have been noticed elsewhere.

Characters of Bacilli.—The bacilli vary in length from 1·5 to 3·5 μ and average rather over 2 μ. About one-tenth are beaded or stained irregularly, and about one-fifth are curved.

SUMMARY.

Progressive tuberculosis of a moderately severe type, associated with fibrin formation. Bacilli not very numerous. The lesions are more severe than those found in Calf 48.

VIRUS—B I.

Animal Inoculated.—Cow 74.

Cow 74 received an intramammary inoculation, into two quarters, with an emulsion of the udder of Cow 40, in which very few bacilli were found. Cow 74 died 299 days after inoculation.

Histological Changes.—The lung tissue is to a great extent replaced with large, irregular, caseous tracts, and the intervening areas are in a highly catarrhal condition.

Distribution and Characters of Bacilli.—The caseous areas contain very large numbers of tubercle bacilli, which are visible everywhere with a low power. Bacilli are also found amongst the alveolar epithelial cells in the rest of the tissue. The bacilli vary in length from 1 to 4μ, the average being about 2μ. The longer forms are generally curved.

SUMMARY.

The lungs are acutely infected and contain enormous numbers of tubercle bacilli. It is interesting to note that this condition was brought about 299 days after intramammary inoculation with a small number of bacilli.

VIRUS.—B I.

Animal Inoculated.—Calf 132.

Calf 132 was inoculated subcutaneously with an emulsion from organs of Monkey 62, estimated to contain about 1,000,000 bacilli. Monkey 62 had been fed with the milk of Cow 74. Calf 132 died 35 days after inoculation.

General Histological Characters.—The portions of the lungs examined exhibit intense vascular engorgement and œdema, and are packed with small consolidated areas which are more or less discrete, but are irregular in outline, and tend to become confluent. The centre of these areas contains a good deal of nuclear débris and cell infiltration. There is no evidence of fibrous tissue stroma either surrounding or within the nodules.

Fibrin.—Fibrin is present in large amount in the consolidated patches. Early fibrin is also present in some of the blood-vessels and within and surrounding the alveolar walls. It also occurs in some patches of subpleural vascular engorgement.

Minute Characters of Cells.—There is a faint indication of plasma cells, but these cells are not satisfactorily demonstrable in this tissue by Pappenheim's method. This method of staining is frequently a failure with the tissues of animals which have been found dead. Eosinophil cells, most of them multinuclear, are present in large numbers, both in the consolidated areas and in the rest of the tissue; granulation is present in most of them, but is, in the majority of cases, indistinct. The consolidated foci do not stain very well; they appear to consist mainly of a mixture of disintegrating epithelial cells, leucocytes, lymphocytes, and indistinguishable cell débris.

Distribution of Bacilli.—Tubercle bacilli are present in enormous numbers; in many of the consolidated foci they are packed so closely as to be visible with a low power. None have been found actually within intact blood-vessels or capillaries, but with the exception of these situations they occur throughout the tissue. A good many bacilli are intracellular; the protoplasm of some cells, notably desquamated epithelial cells, is filled with bacilli.

Characters of Bacilli.—The bacilli are from 2 to 3μ in length, rather more are straight than curved, and not many are beaded.

SUMMARY.

Acute miliary tuberculosis; abundant fibrin formation; dense multiplication of bacilli.

VIRUS.—B II.

Animal Inoculated.—Heifer 14.

Heifer 14 was inoculated subcutaneously with an emulsion of the original material and was killed 35 days afterwards.

General Histological Characters.—Consolidated patches of irregular shape and varying size are found throughout the lung substance. They contain a good deal of cell infiltration and a good deal of adult fibrous tissue, but have not a definite boundary zone of fibrous tissue. Caseation is not very advanced, and is confined to the larger patches. The consolidated areas are highly vascular; no giant cells are noticed within them. The smaller patches are completely isolated; the larger exhibit some tendency to merge into one another and into the surrounding lung tissue, the alveoli of which are filled with a coagulated deposit. The rest of the lung tissue is catarrhal in some places and markedly emphysematous in others. Beneath the pleura small nodules are found, isolated from the lung substance and richly supplied with blood vessels.

Fibrin.—The consolidated patches contain a considerable amount of fibrin, not, for the most part, recent.

Minute Characters of Cells.—There is evidence of plasma cells round the margin of the nodules and round some of the bronchioles, but Pappenheim's stain is not very successfull. Multinuclear leucocytes without very distinct granulation are abundant amongst the consolidated tissue. These areas are further characterised by the presence of numerous blood capillaries and groups of fibroblasts growing between them. Further details are obscured by the copious fibrinous deposit.

Distribution of Bacilli.—Tubercle bacilli are present in very considerable numbers in all the consolidated foci.

SUMMARY.

Progressive tuberculosis. The tubercles, though for the most part isolated, exhibit well marked fibrin formation and contain numerous bacilli.

VIRUS.—B III.

Animal Inoculated.—Cow 68.

Cow 68 received an intramammary inoculation with the original material. The dose inoculated into each of two quarters was estimated to contain 14,500,000 bacilli. The animal was killed 48 days after inoculation.

Histological Changes.—Numerous miliary tubercles are scattered throughout the lung substance. The larger of these are caseous, but the smaller, which are more numerous, are in a very early stage of formation and show no sign of caseation. The tubercles are all of irregular outline and show no sign of demarcation from the rest of the tissue.

Distribution of Bacilli.—Tubercle bacilli are present in all the tubercles but are not abundant; they are more numerous in the older foci.

Summary.

An early stage of progressive miliary tuberculosis.

VIRUS.—B III.

Animal Inoculated.—Calf 210.

Calf 210 was inoculated subcutaneously with ·02 mg. of culture of B. III. and was killed 143 days afterwards. The number of bacilli contained in this dose may be regarded, roughly, as from 80 to 100 million.

Histological Changes.—The two specimens examined each contain several small, discrete tubercles which are clearly marked off from the surrounding tissue. The tubercles show a broad periphery of lymphocytes and fibroblasts; internal to this layer there are some giant cells; the central areas are caseous and often contain some calcareous deposit.

Distribution of Bacilli.—Bacilli are only found within the caseous and calcareous material. In these situations they are fairly numerous.

Summary.

The lung contains many small tubercles of a chronic type. These are obviously quiescent, but still contain tubercle bacilli.

VIRUS.—B IV.

Animal Inoculated.—Calf 140.

Calf 140 (bull—10½ weeks old) received the following subcutaneous inoculations. (1) 100,000 bacilli contained in an emulsion of the tissue of Calf 120; (2) 160 days afterwards, 25 mg. of culture of B IV.; (3) 77 days afterwards, 50 mg. of culture of B IV.; (4) 132 days afterwards, 1 mg. of culture of B IV.; (5) 114 days afterwards, 100 mg. of culture of B IV.; (6) 85 days afterwards, 229 mg. of culture of B IV. The animal was killed in good health 41 days after the last and 609 days after the first inoculation.

Histological Changes.—The blood-capillaries are somewhat dilated and there is a slight thickening of the alveolar walls, there is no histological evidence of tuberculosis.

Distribution of Bacilli.—One small group of bacilli, resembling serum grown bacilli, has been found. There is no histological reaction in the neighbourhood of these bacilli.

Summary.

The absence of tubercular lesions, in spite of the enormous doses inoculated, indicates that the animal had been successfully immunised.*

VIRUS.—B IV.

Animal Inoculated.—Cow 172.

Cow 172 received an intramammary inoculation, into each of two quarters, with an emulsion of the tissue of Calf 138. The dose inoculated into each quarter was estimated to contain 14,500,000 bacilli. Cow 172 was killed 392 days after inoculation.

Histological Changes.—There is a slight, irregularly distributed thickening of the alveolar walls and one or two giant cells have been found, but there are no lesions with the definite histological structure of tubercles.

Distribution of Bacilli.—Only three bacilli have been found in several specimens. These are all present in the wall of one alveolus.

Summary.

The amount of infection is extremely slight.

* Several specimens of liver and kidney have also been examined, but neither tubercles nor tubercle bacilli have been found in them.

VIRUS.—B IX.

Animal Fed.—Calf 380.

Calf 380 was fed with 1 mg. of culture of B IX. and was killed 140 days afterwards.

Histological Changes.—Many large tubercles of a chronic type are present. They are extensively caseous and contain small calcareous deposits. Giant cells are very numerous.

Distribution of Bacilli.—Bacilli are present in some of the tubercles, but are scanty.

SUMMARY.

Numerous caseous tubercles of a chronic type are present. They contain few bacilli. It is noteworthy that these lesions were produced by feeding with a small dose of culture.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Cow 63.

Cow 63 was inoculated subcutaneously with an emulsion of bacilli derived from the tissues of Calf 89. The dose was estimated to contain, approximately, 160 million bacilli. Cow 63 died 45 days after inoculation.

General Histological Characters.—Small caseous foci, not sharply circumscribed, but surrounded with some cell infiltration, are found with moderate frequency in the lung substance. They merge into the surrounding tissue, which is œdematous. The amount of tissue destruction is, on the whole, small; much of the lung tissue shows little deviation from the normal. Beneath the pleura are some rather large nodules which contain much fibrous tissue.

Fibrin.—Both old and recent fibrin are well demonstrated in the tubercular nodules; there is also some early fibrin, both intercellular and intravascular, in the rest of the tissue.

Minute Characters of Cells.—Pappenheim's stain for plasma cells is not successful; situated at the margin of the nodules there are some cells, probably of this character, the protoplasm of which stains a faint pink. Oxyphil leucocytes are numerous in the tubercular nodules, and are also fairly abundant in the rest of the tissue; they are almost all granular; the granules of the leucocytes with an irregular, deeply staining nucleus are, as a rule, smaller than those of the cells, both mononuclear and multinuclear with a pale blue nucleus. A few large cells are also present with purple granules. Small round lymphocytes are abundant. There is little evidence of proliferation of the fixed tissue cells.

Distribution of Bacilli.—Tubercle bacilli are found, but are very scanty. In the specimens stained for fibrin, a bacillus is occasionally seen amongst a fibrinous deposit, but many patches of fibrinous deposit occur in which no bacilli are visible.

SUMMARY.

The tubercles have a broad cellular, but not a fibrous margin. There is no indication that they are progressing rapidly. Bacilli are very scanty. Fibrin is abundant, a feature generally associated with acute lesions.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 83.

Calf 83 was inoculated subcutaneously with an emulsion from the tissues of Calf 79, containing approximately 300,000 bacilli. The animal was killed when moribund, 134 days after inoculation.

Portions of lung tissue, a nodule projecting on a pleural surface of the lungs, and a nodule on the parietal pleura have been examined.

General Histological Characters.—The portions of the lung tissue examined are almost completely solidified. They are characterised by cellular infiltration and new connective tissue growth without the formation of any definite structures. Patches of caseous material are present, and there are some calcareous deposits. Irregular patches of lung alveoli are still recognisable amongst the consolidated tissue; a few of these are patent, but most of them are filled with coagulated exudate.

The subpleural nodule contains no evidence of lung tissue, and is of a densely fibrous structure. The central portions are necrotic and calcareous; a few blood-vessels are present, and there is a great deal of cellular infiltration.

The nodule on the parietal pleura is similar in structure, but is not quite so necrotic and rather more vascular; it does not contain any calcareous deposit.

Fibrin.—In the lung tissue patches of fibrin are found at irregular intervals in the areas both of complete and of partial consolidation.

There is no satisfactory evidence of fibrin in the subpleural nodule.

Fibrin is present in the nodule examined from the parietal pleura.

Minute Characters of Cells.—In the consolidated lung substance there is a diffuse proliferation and infiltration of many varieties of cells without the structural formation of tubercles. Large plasma cells are numerous and stain well; they occur throughout the tissue except in the necrotic patches, and are often in process of transition into fibroblasts. Many smaller cells, which are probably "daughter" plasma cells, also occur. Oxyphil leucocytes are present, but are rare; in this respect there is a striking difference between this lung and several of the lungs above recorded, which contain these cells in large numbers. Some of the oxyphil cells found here have very coarse granules; others have no distinct granulation. Small, round lymphocytes are numerous. Connective tissue corpuscles are numerous and show evidence of proliferation; in van Gieson specimens there are also signs of new formation of adult connective tissue fibres. The proliferation of cells which are undoubtedly endothelial is also a prominent feature in these sections; many of them are growing in parallel columns and some formed fused masses suggestive of giant cells. There is desquamation and apparently some proliferation of the cells of the alveolar epithelium. In some cases, but by no means always, these cells can be distinguished from endothelial cells by their more rounded or polygonal cell outline, more rounded nucleus and more conspicuous nucleolus. In Pappenheim specimens the large red nucleoli of epithelial cells are well brought out, though smaller red nucleoli are also to be found in other cells.

In the subpleural nodule on the lungs there is very little elastic tissue. Plasma cells are numerous; many of them

are merging into fibroblasts. The tissue is densely infiltrated with small round lymphocytes and possibly "daughter" plasma cells. Young connective tissue corpuscles are numerous, and there are many cells, particularly in proximity to the necrotic patches, which appear to be of endothelial type. Cells are also frequent, the nuclei of which resemble those of more or less broken-down multinuclear leucocytes; but their protoplasm does not exhibit an oxyphil reaction. In the lung tissue immediately beneath the nodule multinuclear leucocytes with oxyphil protoplasm are present in moderate numbers.

In the growth on the parietal pleura the characters of the cells are the same as in the subpleural nodule already described.

Distribution of Bacilli.—In the consolidated portions of the lungs tubercle bacilli are universally distributed in large numbers. They are most abundant in the areas of commencing necrosis; in other situations they not infrequently occur within or closely applied to tissue cells, cells of the lung parenchyma, endothelial cells, or young connective tissue corpuscles; they are not frequent within leucocytes.

Bacilli are very numerous throughout the subpleural nodule on the lung; a few can be seen in the calcified areas.

Throughout the growth on the parietal pleura bacilli are present in moderate numbers.

Characters of Bacilli.—The bacilli vary in length from 1·5 to 4μ. and average about 2·5μ. About one-third are curved, and about three-fourths are beaded or irregularly stained.

SUMMARY.

Extensive and progressive tuberculosis, with much new tissue formation and pleural outgrowths. Fibrin present. Bacilli abundant.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 85.

Calf 85 was inoculated subperitoneally with an emulsion from the tissues of Calf 79, containing approximately 300,000 bacilli. The animal was killed 84 days after inoculation.

General Histological Characters. — No pathological features are noticed, under a low power, in the lung substance, but the pleura is thickened and a few nodules are found in it, consisting of a loose fibrillar network poor in cells, and not involving the lung substance. They are separated from the latter by a definite fibrous tissue boundary rich in blood-vessels, but their pleural boundary is not marked by a fibrous tissue zone. Some of the nodules are slightly caseous and contain giant cells.

Fibrin.—No fibrin is found either at the pleural margin of the lung or elsewhere.

Minute Characters of Cells.—An occasional plasma cell is found in the alveolar and bronchial walls; none elsewhere. In the peribronchial lymphatic tissue there are very few cells which take the red protoplasmic stain by Pappenheim's method, which characterises the large lymphocytes in lymphatic glands; in the substance of this tissue neither coarsely nor finely granular oxyphil cells are found, but immediately external to the bronchial epithelium an occasional very coarsely granular cell and an occasional ordinary multinuclear leucocyte is to be seen. Multinuclear leucocytes are distributed in moderate numbers throughout the tissue of the lung alveoli. In the thickened pleura and in the subpleural nodules both multinuclear leucocytes and small round lymphocytes occur, in addition to the cells which send out fibrillar processes.

Distribution of Bacilli.—Tubercle bacilli have not been found either in the subpleural nodules or in the lung substance.

SUMMARY.

The lesions are relatively few in number and appear to be retrogressive. No bacilli have been found

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VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 89.

Calf 89 was inoculated intravenously with an emulsion from the tissues of Calf 79, containing, approximately, 300,000 bacilli. The animal was killed, when very ill, 23 days afterwards.

General Histological Characters.—Distributed at frequent intervals throughout the tissue are consolidated areas, irregular in outline and highly vascular. The centre of these areas is filled with cell débris, and is slightly caseous; at their periphery the outlines of the lung alveoli can still be seen, filled with a coagulated deposit. There are catarrhal patches in the rest of the lung tissue, and several areas of collapse.

Fibrin.—No recent fibrin is found, and there is very little evidence of old fibrin in the consolidated patches.

Minute Characters of Cells.—Plasma cells are numerous in the tubercular parts; many of them are passing into fibroblasts. Multinuclear leucocytes occur plentifully; in the majority of them the granulation is indistinct. In addition to the red blood corpuscles contained within capillaries, there are large numbers of red blood corpuscles lying free throughout the consolidated areas. In these areas the alveolar epithelial cells have for the most part disappeared, but there is a good deal of proliferation of endothelial cells. In some places, at the margins of a tubercular focus, the walls of blood capillaries which contain red corpuscles are seen to be proliferating, and in more of less complete continuity with these are seen newly-formed, empty capillary channels.

Distribution and Characters of Bacilli.—Tubercle bacilli are abundant throughout all the tubercular areas. The bacilli vary in length from 1 to 3μ, the average being about 2μ.

SUMMARY.

Acute progressive miliary tuberculosis. Bacilli very abundant.

VIRUS.—H 2. Sp. A.
Animal Inoculated.—Calf 93.

Calf 93 received an intraperitoneal inoculation, estimated to contain about 600,000 bacilli, with an emulsion of the tissues of Calf 79. Calf 93 died 73 days after inoculation.

General Histological Characters.—Gritty portions were felt in the lung tissues, and decalcification was necessary. The lung substance shows very considerable catarrhal change, with swollen alveolar walls, desquamated epithelium, and a coagulated exudate filling many of the alveoli. It also contains large areas of consolidated tissue; these have not very definite boundaries, but merge into the surrounding tissue; in van Gieson specimens they are seen to be permeated with a good deal of fibrous tissue; they are partly caseous, highly vascular, and contain here and there masses of small round cells. Projecting from the surface of the lung are prominent out-growths which on section are found microscopically to contain a dense network of fibrous tissue, and to be partially necrotic in the centre. The interstices of the fibrous tissue stroma are, particularly in the outer portions of the nodules, packed with an enormous number of blood channels engorged with red corpuscles. On part of the free surface of the nodules there is, external to the fibrous structure, an outer layer of loose fibrillar tissue, containing very few cells and consisting mainly of a widely-meshed stroma which stains red in van Gieson preparations. In this stroma, also, engorged blood-vessels are numerous and very conspicuous.

Fibrin.—In the lung substance old fibrin is found in the old tubercular foci, and early fibrin in the rest of the tissue, both amongst the tissue cells and within some of the blood-vessels. Similarly, in the subpleural nodules old fibrin is found in the necrotic areas, and delicate strands of early fibrin are present in the rest of the nodule; the adjacent lung substance also contains early fibrin. Kochel's method gives an exceptionally good differentiation of fibrin in all the specimens taken from this lung.

Minute Characters of Cells.—The decalcification which was requisite has interfered with the protoplasmic staining reactions both in Pappenheim and in eosin and methylene blue specimens, and it is not possible to determine the number of plasma cells or oxyphil cells present. Small round lymphocytes are exceptionally numerous. Connective tissue corpuscles are also numerous, and there is a good deal of desquamation of epithelial cells, but no evidence of their proliferation. Elastic tissue is conspicuous in the basal third of a subpleural nodule examined, but not in the distal portion.

Distribution of Bacilli.—All the consolidated parts of the lung tissue, except completely necrotic areas, are swarming with bacilli; clusters of bacilli are visible with a low power in all parts of the field. The subpleural nodule examined contains bacilli in even greater numbers; they form colonies which are more or less continuous with one another throughout the nodule. Neither in the lung nor in the subpleural nodule are bacilli found within intact blood-vessels.

SUMMARY.

Advanced, progressive tuberculosis, with calcareous deposits in the old foci. Well-marked fibrin formation. Bacilli very abundant.

VIRUS.—H 2. Sp. A.
Animal Inoculated.—Calf 153.

Calf 153 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated with the tissues of Heifer 13. Calf 153 was killed, when very ill, 39 days after inoculation.

General Histological Characters.—Small isolated tubercles without any fibrous tissue demarcation and slightly caseous in their centres are distributed in large numbers throughout the lung tissue, and also occur as projections beneath the pleura.

Fibrin.—Small deposits, which appear to be old fibrin, are found in a few of the consolidated foci.

Minute Characters of Cells.—Plasma cells are present at the margin of the consolidated areas and round some of the bronchioles, but do not stain well. Multinuclear leucocytes are fairly numerous in the tubercular nodules; many of them are coarsely granular. A considerable proportion of the leucocytes which occur in the rest of the lung tissue are also coarsely granular. Small round lymphocytes are present in moderate numbers in the tubercles. Both leucocytes and lymphocytes occur as an infiltration amongst cells of an epithelioid type. These last cells do not stain well, and it is impossible to say how many of them are remains of alveolar epithelial cells and how many are of endothelial origin. Giant cells are occasionally found. Definitely recognisable young connective tissue corpuscles are not numerous.

Distribution of Bacilli.—Tubercle bacilli are present in the consolidated foci, but are not very numerous.

SUMMARY.

Many small, isolated, but progressive tubercles are present. Bacilli are not very numerous.

VIRUS.—H 7. C.M.
Animal Inoculated.—Calf 5.

Calf 5 was inoculated subcutaneously with an emulsion of the original material and was killed 109 days afterwards.

Histological Changes.—The lung is in a semi-solid condition and contains many large caseous tracts. These caseous areas have no definite outline, but are evidently spreading into the neighbouring alveoli, which are partially consolidated.

Distribution of Bacilli.—Bacilli are present in the caseous areas, but are rare.

SUMMARY.

Advanced, progressive tuberculosis of the lungs. Bacilli rare.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 101.

Calf 101 was inoculated intravenously with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli. The animal was killed when moribund, 41 days after inoculation.

General Histological Characters.—The portions of the lungs examined are in a condition of acute catarrh, and are also occupied with very numerous, irregular patches of consolidated tissue which are generally small in size, but are tending to become confluent. Within them are relatively extensive areas of caseation; surrounding and partially invading them is a good deal of cellular infiltration; there is also an irregular increase of fibrous tissue which ramifies in all directions, and has no tendency to circumscribe the consolidated patches. Extending from these completely consolidated areas there is a widespread interstitial infiltration of the surrounding alveoli, which are filled with catarrhal deposits.

Fibrin.—Fibrin is present in the definitely tubercular patches in considerable amount, and, to a less extent, in the surrounding tissue.

Minute Characters of Cells.—Plasma cells are numerous and stain well. They occur at the periphery of tubercular foci, round the bronchioles, round the blood-vessels, and form the most prominent constituent of the interstitial tissue which is invading the lung alveoli. Cells of this type also occur, but only very scantily, in the patches of peribronchial lymphatic tissue which are not evidently tubercular; there is no indication that the plasma cells found in the rest of the tissue are derived from this source. Cells with the typical staining reaction of large plasma cells are not found in the blood-vessels, although a few lymphocytes are present in this situation with a relatively large round nucleus and a narrow margin of protoplasm which stains a faint pink. The majority of the oxyphil leucocytes present are multinuclear, and without coarse granulation. These cells are abundant within the bronchioles and lung alveoli, but are not particularly numerous in the tubercular areas. Fibroblasts are abundant round the margins of the tubercular foci, and amongst the infiltrated alveoli. There is extensive desquamation of the alveolar epithelial cells, but no evidence of their proliferation. In many of the tubercular foci they have entirely disappeared. In the earlier consolidated foci there appears to be a proliferation of endothelial cells.

Distribution and Characters of Bacilli.—In the histologically tubercular areas tubercle bacilli are plentiful, about fifty in nearly every microscopic field. One or two bacilli have been noted within blood-vessels, but their presence there is so rare that it is probably an accident, due to the preparation of the section. The bacilli vary in length from 1 to 3·5 μ, the average being about 2 μ. Many forms are notably slender.

SUMMARY.

Numerous small disseminated tubercles, tending to become confluent. Fibrin present. Bacilli numerous.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 103.

Calf 103 was inoculated subcutaneously with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli. The animal was killed 130 days afterwards.

General Histological Characters.—In the sections examined the only abnormalities noted with a low power are small subpleural projections which consist of an open fibrillar structure containing several blood-vessels and not many cells.

Fibrin.—There is no evidence of fibrin either in the subpleural nodules or elsewhere.

Minute Characters of Cells.—No plasma cells are found. In the subpleural nodule finely granular multinuclear leucocytes are found, and also small lymphocytes. The rest of the lung tissue contains an abnormally large number of multinuclear leucocytes; these cells are also present in excessive numbers within the blood-vessels. No oxyphil cells are found in the peribronchial lymphatic tissue.

Distribution of Bacilli.—No tubercle bacilli have been found either in the subpleural projections or elsewhere.

SUMMARY.

No definite evidence of tuberculosis in the specimens examined. In marked contrast to the severe infection produced by intravenous inoculation of the same dose.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 105.

Calf 105 was inoculated subcutaneously with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli, and was killed, when moribund, 47 days afterwards.

Histological Changes.—The tissue is occupied by innumerable miliary tubercles which are becoming confluent. Much of the tissue external to these tubercles is in a semi-solid condition. The larger tubercles are extensively caseous.

Distribution of Bacilli.—Bacilli are present in the affected areas in moderate numbers, but not so plentifully as in Calf 101.

SUMMARY.

The condition of the lungs is similar to that produced by intravenous inoculation with the same dose, but bacilli are not quite so plentiful.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 109.

Calf 109 received an intraperitoneal inoculation with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli, and died 40 days afterwards.

Histological Changes.—The tissue is in a condition of severe catarrh and contains many miliary tubercles in an early stage of formation.

Distribution of Bacilli.—The tubercles contain numerous bacilli.

SUMMARY.

Early miliary tuberculosis.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 141.

Calf 141 was inoculated subcutaneously with an emulsion from the tissues of Cow 73 estimated to contain about 250,000 bacilli. The animal was killed 53 days after inoculation.

General Histological Characters.—The general appearance of the substance of the lung is very similar to that of Calf 101, but the areas of consolidation and caseation are rather more extensive, and in the centre of some of the caseous areas there is a small calcareous nodule. Projecting from the pleura, and attached to the lung by a broad base, are outgrowths of tissue which is on the whole dense, is surrounded and permeated by fibrous tissue, and is centrally caseous. In many parts the periphery of these outgrowths consists of a loose network of fibrillar tissue containing few cells; this type of tissue often projects outwards in delicate tuft-like elongations which contain blood-vessels.

Fibrin.—The distribution of fibrin is similar to that in Calf 101.

Minute Character of Cells.—Plasma cells are present in considerable numbers, and have the same distribution as in Calf 101, though not so numerous and not staining so well; many of them are becoming narrowed and elongated into fibroblasts. Oxyphil cells, for the most part finely granular, are more abundant in the areas of tubercular consolidation than in Calf 101, and small lymphocytes are also rather more abundant. Cells of epithelial and endothelial types exhibit more advanced disintegration.

Distribution of Bacilli.—The distribution of bacilli in the areas of tubercular consolidation is the same as in Calf 101, but they are not more than half as numerous. Many are found in the catarrhal patches filled with coagulated exudate; in these situations they are much more numerous than in Calf 101.

SUMMARY.

The lesions are similar, on the whole, to those produced in Calf 101 by intravenous inoculation with this virus

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VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 305.

Calf 305 was inoculated intravenously with an emulsion from the tissues of guinea-pigs which had been inoculated from Calf 177. The dose inoculated was estimated to contain, approximately, 730,000,000 bacilli. Calf 305 was killed, when moribund, 26 days after inoculation.

Histological Changes.—One specimen of the lung shows complete consolidation. In another specimen there is very marked thickening and infiltration of the alveolar walls; this process is uniformly distributed throughout the specimen, but has not advanced to complete consolidation. The consolidated areas contain a large number of multinuclear leucocytes and show a slight tendency to necrosis. They are nowhere definitely caseous, nor do they contain giant cells. There are slight traces of fibrin.

Distribution and Characters of Bacilli.—Bacilli are numerous throughout the tissue. Many of them are notably long (3–4μ) and curved. Some of them appear to have multiplied within desquamated epithelial cells; they are frequently contained within endothelial cells, and occasionally within multinuclear leucocytes.

SUMMARY.

The lungs are in a condition of solidification, due to the presence of tubercle bacilli. The lesions have not the irregular patchy distribution which is typical of ordinary tubercular pneumonia.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 361.

Calf 361 was inoculated intravenously with an emulsion, estimated to contain 41,500,000 bacilli, from the tissues of Calf 275, and was killed 76 days afterwards.

Histological Changes.—The lung is crowded with miliary tubercles. The lesions are centrally caseous, often contain giant cells, and have a broad peripheral zone. Though often closely set together, the tubercles are generally distinguishable from one another, and, judging by the density of their peripheral zones, do not appear to be actively progressive. The periphery consists of fibroblasts, an abundant infiltration of plasma cells, and numerous lymphocytes. Oxyphil leucocytes are present in most of the tubercles, but are nowhere numerous.

Distribution of Bacilli.—Tubercle bacilli are to be found in many of the tubercles, but are scanty.

SUMMARY.

The lung is crowded with typical caseous tubercles which are of the chronic type and contain few bacilli.

VIRUS.—H 9. C.T.

Animal Inoculated.—Calf 185.

Calf 185 was inoculated subcutaneously with ·75 cc. of wet bacilli grown on glycerinated broth. The culture was four and a half months old. The animal was killed 40 days afterwards.

General Histological Characters.—The lungs exhibit small tubercles, not numerous but pretty regularly distributed, both within their substance and beneath the pleura. Apart from these foci no pathological change is noticeable under a low power. The tubercles, though isolated and fairly regular in outline, are not circumscribed by fibrous tissue. They contain giant cells, and the larger of them are caseous in the centre.

Fibrin.—No fibrin is present.

Minute Characters of Cells.—There is hardly any indication of plasma cells. The tubercles contain large numbers of oxyphil leucocytes; most of them are multinuclear and are granular; the granules are fairly definite and of moderate size. Leucocytes, exhibiting the same characters, are found in considerable numbers in the rest of the tissue, with the exception of the lymphoid patches, where they do not occur. An occasional cell larger in size, with a more regular nucleus, and with purple granules, is found in the non-tubercular areas. The characters of the other cells forming the tubercles cannot be made out with certainty. Many of them resemble ordinary alveolar epithelial cells.

Distribution of Bacilli.—A few bacilli are present in the tubercles.

SUMMARY.

Small tubercles, poor in bacilli, and tending to caseate.

VIRUS.—H 10. B.S.

Animal Inoculated.—Heifer 81.

Heifer 81 was inoculated subcutaneously with an emulsion of the original material and was killed 132 days afterwards.

General Histological Characters.—In the lung are found small numbers of isolated tubercular nodules, which are definitely circumscribed by a broad fibrous tissue zone, exhibit commencing caseation in the centre, contain a few large giant cells, and are well supplied with blood-vessels. The lesions in these lungs are very similar to those described above in Heifer 30, which was inoculated with B1.

Fibrin.—There is no clear evidence of fibrin.

Minute Characters of Cells.—Plasma cells, many of them passing into fibroblasts, are found round the outer portions of the nodules. As in Heifer 30, the peripheral portions of the tubercles contain an enormous number of oxyphil leucocytes, filled with rather large and very distinct granules. The same varieties of these cells are present as those already described in Heifer 30, and the relative numbers of each and their distribution are also the same. Small round lymphocytes with a deeply stained nucleus are much fewer than the leucocytes. The distribution and character of the epithelioid cells is the same as in Heifer 30.

Distribution of Bacilli.—Tubercle bacilli are found within the nodules, near their margin, but are very rare; none have been seen in the caseous portions of the nodules nor in other parts of the lung tissue.

SUMMARY.

Tubercles of the chronic type, similar to those produced in Heifers 26 and 30 after inoculation with a bovine virus.

VIRUS.—H 10. B.S.

Animal Inoculated.—Calf 191.

Calf 191 was inoculated subcutaneously with an emulsion from the tissues of Calf 35 estimated to contain about 2,500,000 bacilli, and was killed, when moribund, 40 days afterwards.

Histological Changes.—The lungs have the patchy consolidation typical of tubercular pneumonia. The lesions are irregular in outline, obviously progressive, and in an advanced stage of caseation.

Distribution of Bacilli.—Bacilli are present in all the lesions, but are not numerous.

SUMMARY.

Advanced, progressive, caseating pneumonia. Bacilli scanty.

VIRUS.—H 12. H.N.

Animal Inoculated.—Calf 319.

Calf 319 was inoculated intravenously with an emulsion of tissue from guinea-pigs which had been inoculated from Calf 189. The dose inoculated was estimated to contain over 100,000,000 bacilli. Calf 319 was killed, when moribund, 57 days after inoculation.

Histological Changes.—The tissue is densely packed with small miliary tubercles. The tubercles are crowded with multinuclear leucocytes; some of the foci are commencing to break down in the centre, but most of them have not advanced to necrosis. In most places the tubercles are becoming confluent. No fibrin is present.

Distribution and Characters of Bacilli.—The entire tissue is swarming with bacilli which, in all except the smallest foci, have grown into dense masses forming conspicuous objects under a low power. The bacilli vary in size and shape; short (1–1·5μ) straight forms are numerous in areas of dense multiplication. Long and curved forms (3–4·5μ) are also numerous.

SUMMARY.

Miliary tuberculosis advancing to general consolidation. Enormous numbers of bacilli. No fibrin. Amount of tissue destruction small relatively to the large number of bacilli present.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 301

Calf 301 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated from Calf 129 The dose was estimated to contain over 540 000,000 bacilli. Calf 301 was killed, when very ill, 33 days after inocu ation.

Histological Changes.—Specimens from three different portions of lung have been examined. Two of these are completely solidified; in the third there are still some air-containing alveoli left. Most of the lesions are caseous, and contain much cellular débris. The general appearance is typical of an acute tubercular type of pneumonia. There is an abundant deposit of fibrin.

Distribution of Bacilli.—Bacilli are abundant throughout the tissue and have grown into conspicuous masses in the older lesions.

Summary.

Acute, rapidly progressive tuberculosis, associated with fibrin formation and the presence of large number of bacilli.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 325.

Calf 325 was inoculated subcutaneously with an emulsion from the tissues of Calf 301 containing, approximately 500,000,000 bacilli. Calf 325 was killed, when moribund, 24 days afterwards.

Histological Changes.—The specimen of lung is completely solid and is extensively caseous. It contains a fibrinous deposit, and is evidently undergoing rapid disintegration.

Distribution of Bacilli.—Bacilli are very abundant throughout the specimen.

Summary.

Highly acute tuberculosis. Extensive tissue destruction. Bacilli numerous.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 121.

Calf 121 was inoculated subcutaneously with an emulsion of the original material estimated to contain over 360,000,000 bacilli. The animal was killed, when ill, 36 days afterwards.

General Histological Characters.—The lung tissue is closely packed with consolidated foci, most of which are caseous in the centre. Some of these foci are partially circumscribed with fibrous tissue, but the great majority of them are not. The foci appear to be spreading, and are to a large extent confluent with their neighbours. In the rest of the lung tissue are found catarrhal change and areas of collapse.

Fibrin.—There is a copious fibrinous exudate in many of the consolidated patches. Both old and recent fibrin is found.

Minute Characters of Cells.—Plasma cells appear to be present in fairly large numbers, and to be generally distributed, but their protoplasm only takes a faint pink stain. Multinuclear leucocytes, mostly of the finely granular type, are abundant in the bronchioles, the alveoli, the consolidated tissue, and the alveolar walls. The number of white corpuscles within the blood-vessels is abnormally large. Small lymphocytes are also present in considerable numbers in the tissue. Most of the epithelial cells of the alveoli have desquamated; but many of them still retain their staining properties. There is a good deal of proliferation of cells of connective tissue and endothelial types, but the proliferation is rapidly giving place to necrosis. The process of new tissue formation seems to be arrested, and to give way to necrotic change, shortly before the formation of completely organised tubercles.

Distribution of Bacilli.—Tubercle bacilli are found in fairly large numbers throughout the consolidated areas.

Characters of Bacilli.—The bacilli vary in length from 1·5 to 3·5μ and average about 2·5μ. About one-third are curved and a rather smaller proportion are either beaded or irregularly stained.

Summary.

The tissue is packed with miliary tubercles which are becoming confluent. Fibrin is present. Bacilli are fairly numerous.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 125.

Calf 125 was inoculated subcutaneously with the same dose of the same material as Calf 121 and was killed 23 days afterwards.

General Histological Characters.—The lesions are similar to those of Calf 121, but not quite so advanced. There is not so much caseation in the tubercular areas; these are smaller on the whole, and not so many of them are confluent.

Fibrin.—Both old and recent fibrin are present.

Minute Characters of Cells.—Plasma cells are numerous and stain rather better than in Calf 121. Multinuclear leucocytes of the finely granular type are even more numerous than in Calf 121, and are particularly abundant in the consolidated areas. In these situations they are frequently aggregated in masses, situated centrally; in the areas which show no sign of caseation, the protoplasm

of the leucocytes stains well ; in the areas of commencing caseation their nuclei, as well as their protoplasm, are disintegrated. In other respects the cells observed in these lungs are similar to those recorded in Calf 121.

Distribution of Bacilli.—The tubercle bacilli are distributed in about the same numbers as in Calf 121, but more of them appear to be intracellular. Many of the cells containing bacilli are undoubtedly epithelial, and it is not evident that the number of intracellular bacilli is greater where aggregations of leucocytes occur, nor that many of the cells containing the bacilli are leucocytes.

SUMMARY.

The lesions are similar to those found in Calf 121 but not quite so advanced.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 197.

Heifer 197 was inoculated subcutaneously with an emulsion of the tissues of Calf 121 containing, approximately, 85,500,000 bacilli. Heifer 197 was killed, when very ill, 51 days afterwards.

Histological Changes.—The tissue contains large irregular, semi-confluent, caseating areas, between which a few groups of air-containing alveoli are still to be found.

Distribution of Bacilli.—Bacilli are present in small numbers.

SUMMARY.

Exten ive, progressive, caseous pneumonia. Bacilli not numerous.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 243.

Heifer 243 was inoculated subcutaneously with an emulsion, estimated to contain about 4,500,000 bacilli from the tissues of Heifer 197, and was killed, when very ill, 61 days afterwards.

Histological Changes.—The tissue is occupied with large irregular, solidified areas in an advanced stage of caseation ; between these are found tracts of alveoli which are often filled with a coagulated exudate.

Distribution of Bacilli.—Bacilli are numerous within some parts of the caseous material, but in other situations are scanty.

SUMMARY.

Progressive consolidation, with advanced caseation.

VIRUS.—H 16. J.H.

Animal Inoculated.—Heifer 245.

Heifer 245 was inoculated subcutaneously with an emulsion, estimated to contain about 157,000,000 bacilli, of the organs of Rabbit 66 (which had been inoculated from Calf 355), and was killed 209 days afterwards. The animal gave birth to a calf 16 days before being killed.

Histological Changes.—The specimen contains a group of caseous nodules which are partly within the lung substance and partly project beneath the pleura. The nodules are completely caseous in the centre ; at their margins they contain many large giant cells. They are marked off from the adjacent lung tissue, which is normal, by a well marked layer, consisting largely of fibroblasts.

Distribution of Bacilli.—Bacilli are scanty, but are present at the periphery as well as in the centre of the nodules.

SUMMARY.

The lung contains groups of circumscribed, caseous tubercles in which bacilli are scanty. The tissue external to these tubercles is normal.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 273.

Calf 273 was inoculated subcutaneously with an emulsion from the tissues of Calf 187 containing, approximately, 11,000,000 bacilli. Calf 273 was killed 89 days afterwards.

Histological Changes.—Large tubercular nodules, which are extensively caseous, are present. Giant cells are present at their periphery and they are partially bounded by a fibrous zone, but immediately external to this, earlier tubercles are found in process of formation. The lesions therefore appear to be progressive. No fibrin is to be found.

Distribution of Bacilli.—Bacilli are rare.

SUMMARY.

Caseating tuberculosis, of the chronic, slowly progressive type. Bacilli rare.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 281.

Calf 281 was inoculated subcutaneously with an emulsion of the tissues of Calf 355 containing, approximately, 11,000,000 bacilli. Calf 281 was killed, when ill, 54 days afterwards.

Histological Changes.—The tissue contains numerous tubercles. Some of the older and larger are partially marked off from the surrounding tissue by a layer of fibroblasts; but in the younger tubercles, which are more numerous and are quite irregular in outline, this demarcating layer is absent. The proliferation of endothelial cells in the tubercles and the formation of new blood capillaries is notable. Plasma cells are also very numerous. Oxyphil leucocytes are abundant and many of them possess a coarse granulation. No giant cells have been noted. The central parts of the tubercular areas are caseous. There is a well-marked fibrinous deposit.

Distribution of Bacilli.—Tubercle bacilli are moderately numerous, particularly in the older lesions, but nowhere form large groups.

SUMMARY.

The lesions are numerous, progressive, and moderately acute in type. Tubercle bacilli are moderately numerous.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 355.

Calf 355 was inoculated subcutaneously with an emulsion of the tissues of Calf 273 containing, approximately 3,000,000 bacilli. Calf 355 was killed when very ill, 43 days afterwards.

Histological Changes.—The specimen contains numerous tubercles. These are of irregular outline and are not surrounded by fibroblasts. The larger of them are caseous. The tubercles show marked proliferation of cells of endothelial type and are infiltrated with lymphocytes and plasma cells.

Distribution of Bacilli.—Bacilli are present, in somewhat small numbers, in all the lesions.

SUMMARY.

Disseminated tuberculosis of a progressive type. The lesions do not contain numerous bacilli and are not characteristic, histologically, of a very acute infection.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 529.

Calf 529 was inoculated intravenously with an emulsion from the tissues of Calf 539 containing, approximately, 1,000,000,000 bacilli. Calf 529 died 13 days afterwards.

Histological Changes.—In one specimen the tissue is in a condition of nearly complete consolidation; the outlines of the alveoli can still be made out, but there is very little air-containing space left. In a specimen from another part of the lung, the general condition is the same, but the process is less advanced and the air spaces are more numerous. The whole tissue is densely infiltrated with multinuclear leucocytes of the finely granular variety. The larger consolidated foci are just beginning to break down, but there are no definitely caseous tracts. Fine fibrin filaments are found in many situations, but there is nowhere a copious exudate of fibrin.

Distribution and Characters of Bacilli.—Tubercle bacilli are present in enormous numbers throughout the tissue, forming everywhere red groups conspicuous under a low magnification. They frequently occur within blood-vessels. The bacilli have obviously multiplied with great rapidity. Short, straight forms (about 1μ) are more numerous than long, curved forms (3–$4 \cdot 5\mu$).

SUMMARY.

Pneumonic consolidation, due to the presence of enormous numbers of tubercle bacilli.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 555.

Calf 555 was inoculated subcutaneously with an emulsion from the tissues of Calf 553 containing, approximately, 150,000,000 bacilli. Calf 555 was killed when moribund, 58 days afterwards.

Histological Changes.—The condition of the lungs is typical of highly acute, tubercular pneumonia, and is identical with the condition produced by the inoculation of 50 mg. of culture of highly virulent viruses. There is an abundant deposit of fibrin.

Distribution of Bacilli.—Tubercle bacilli are very numerous throughout the tissue.

SUMMARY.

Identical with the condition produced by inoculation of 50 mg. of a highly virulent culture.

VIRUS.—H 17. Sp. B.
Animal Inoculated.—Calf 607.

Calf 607 (a bull, 9 months old) was inoculated intravenously with 90 mg. of culture isolated from Calf 339, and killed, when in good health, 48 days afterwards.

Histological Changes.—Two portions of the lungs have been examined. They both show a considerable amount of irregular thickening of the alveolar walls, but no tubercles.

Distribution of Bacilli.—Tubercle bacilli are extremely rare. In one specimen only have a few been found.

Summary.

The specimens show that in this animal (a bull-calf 9 months old) an enormous dose of culture, intravenously inoculated, had failed to produce tuberculosis, and that the bacilli injected have almost all disappeared.

VIRUS.—H 17. Sp. B.
Animal Inoculated.—Calf 685.

Calf 685 was inoculated intravenously with 10 mg. of culture isolated from Calf 339, and killed, when in good health, 64 days after inoculation.

Histological Changes.—The tissue shows some general thickening of the alveolar walls, and also contains a few tubercles. One of these tubercles is about the size of the microscopic field (low power); it is centrally caseous, and is circumscribed with small round cells and fibroblasts. Another, smaller, tubercle is not caseous, but is filled with cells which are mainly lymphocytes.

Distribution and Characters of Bacilli.—In the first-mentioned tubercle about forty or fifty bacilli are present; many of them are rather long, and are irregularly stained. In the smaller, non-caseous tubercle mentioned above, no bacilli have been found. In one situation a fragment of culture is found enclosed within a giant cell; all the bacilli forming the culture mass are like serum bacilli, and show no sign of multiplication.

Summary.

A few small tubercles, of a retrogressive character, are present.

VIRUS.—H 18. T.T.
Animal Inoculated.—Calf 165.

Calf 165 was inoculated subcutaneously with an emulsion, estimated to contain about 4,400,000 bacilli of the original material, and was killed 57 days afterwards.

Histological Changes.—Specimens from two different parts of the lung have been examined. They both contain groups of small tubercles, which are slightly caseous, are surrounded by a broad fibrous zone, and contain numerous giant cells.

Distribution of Bacilli.—Each tubercle contains a few bacilli.

Summary.

The lung contains many small tubercles which are of the chronic type and exhibit marked evidence of progressively conservative change.

VIRUS.—H 18. T.T.
Animal Inoculated.—Calf 405.

Calf 405 was inoculated subcutaneously with an emulsion from the organs of Guinea-pig 173, containing about 270,000,000 bacilli; this guinea-pig was one of a series inoculated from Calf 131. Calf 405 was killed 55 days afterwards.

Histological Changes.—The alveolar walls are thickened, and throughout the specimen there are numerous patches of consolidation which vary in size; the smaller are not larger than a single alveolus, whilst the larger are equivalent to a group of half a dozen or more. These patches are of irregular shape and have not the formation of definite tubercles. They are not caseous and contain no giant cells; the cells composing them consist mainly of fibroblasts and lymphocytes, amongst which are a relatively smaller number of leucocytes. No fibrin is present.

Distribution of Bacilli.—In most of the consolidated patches a few tubercle bacilli are to be found.

Summary.

The condition of the lungs indicates that there has been an invasion of tubercle bacilli which has been to a large extent overcome, and is being followed by a process of diffuse fibroid change.

VIRUS.—H 19. S.W.

Animal Inoculated.—Calf 159.

Calf 159 was inoculated subcutaneously with an emulsion estimated to contain 1,500,000 bacilli of the original material, and was killed 50 days afterwards.

Histological Changes.—The lung contains large ramifying tubercular tracts which are extensively caseous. Traces of old fibrin are present.

Distribution of Bacilli.—Bacilli are moderately numerous in the lesions.

SUMMARY.

Progressive tuberculosis.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 399.

Calf 399 was inoculated intravenously with an emulsion of the lesions of Guinea-pig 200, containing over 700,000,000 bacilli. This guinea-pig was one of a series inoculated from Calf 291. Calf 399 was killed when very ill, 34 days afterwards.

Histological Changes.—The alveolar walls are greatly thickened and infiltrated, and the general condition of the tissue is one of partial but progressive consolidation. The lesions are not necrotic and do not present any appearance which is histologically typical of tuberculosis. Lymphocytes are present in the cellular infiltration as well as multinuclear leucocytes; of the latter, some are coarsely granular. No fibrin is present.

Distribution and Characters of Bacilli.—The tissue is everywhere swarming with tubercle bacilli, which vary in length from 2 to 4μ, the average being about 3μ.

SUMMARY.

Pneumonic consolidation, due to the presence of enormous numbers of tubercle bacilli. No fibrin is present.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 749.

Calf 749 was inoculated intravenously with 46 mg. of culture isolated from Calf 293, and killed when very ill, 24 days afterwards.

Histological Changes.—One specimen shows a complete pneumonic consolidation, without any caseous areas or other histological indications of tuberculosis. Another specimen, from a different part of the lung, shows a rather less advanced stage of the same condition, the lesions again being not typical of tuberculosis. No fibrin is demonstrable.

Distribution and Character of Bacilli.—Bacilli are distributed in fairly large numbers throughout the tissue. The greater number of them are long (3–4μ) and generally curved.

SUMMARY.

Pneumonic consolidation, due to the presence of tubercle bacilli.

VIRUS.—H 23. J.P.

Animal Inoculated.—Calf 365.

Calf 365 was inoculated subcutaneously with an emulsion of originial material estimated to contain about 100 million bacilli. The animal was killed sixty-one days afterwards.

General Histological Characters.—Small tubercles occur abundantly throughout the tissue. They contain many giant cells, and some of the largest of them are commencing to caseate. There is also a catarrhal thickening of the alveolar walls, and many of the alveoli are occluded, partly with cells and partly with a coagulated deposit. The older tubercles are generally discrete, but the earlier ones are often continuous with the catarrhal patches.

Fibrin.—No fibrin is present.

Minute Characters of Cells.—Oxyphil leucocytes are abundant both in the catarrhal areas and within the tubercles, particularly in the latter situation, where they often form large masses. The general characters of the tubercles indicate an active tissue reaction. Small lymphocytes are plentiful, and plasma cells are particularly numerous and conspicuous both within and surrounding the tubercles and at the periphery of the bronchioles. There is a well-marked proliferation of endothelial cells with formation of new capillary channels. Many of the tubercles are highly vascular. The nuclei of the giant cells suggest an endothelial rather than an epithelial origin. Young fibroblasts are plentiful, but there is hardly any increase of adult fibrous tissue.

Distribution of Bacilli.—Tubercle bacilli are rare. A few are found within definitely formed tubercles, but none elsewhere.

SUMMARY.

The lesions are chronic rather than acute in type, and if the animal had been allowed to live longer it is probable that retrogressive changes would have become much more pronounced.

VIRUS.—H 23. J.P.
Animal Inoculated.—Calf 441.

Calf 441 was inoculated intravenously with an emulsion of the tissues of Calf 345, containing over 13,000,000 bacilli. Calf 441 was killed 102 days afterwards. It was found at the post-mortem examination that part of the inoculated material had lodged in the wall of the vein and amongst the surrounding tissue.

Histological Changes.—The specimen examined contains a group of small tubercles, each of which is centrally caseous and is surrounded by a broad zone of fibroblasts and lymphocytes. There are also patches where the alveolar walls are greatly thickened and contain an increase of the fibrous elements and a large number of lymphocytes and plasma cells. These areas have not the histological appearance of definite tubercles

Distribution of Bacilli.—Bacilli are very rare, but a few have been found in the caseous parts of the tubercles.

SUMMARY.

Numerous, small, retrogressive lesions containing very few bacilli.

VIRUS.—H 25. A.T.
Animal Inoculated.—Calf 551.

Calf 551 (a bull-calf 10 months old) was inoculated intravenously with 100 mg. of culture isolated from Calf 417, and died 21 days afterwards.

Histological Changes.—The tissue exhibits acute catarrhal change (vascular congestion and desquamation of epithelial cells) but no definite tubercles. Early fibrin is present beneath the pleura, amongst the lung parenchyma, and in the blood-vessels.

Distribution and Characters of Bacilli.—Tubercle bacilli are abundant throughout the tissue and are present within blood-vessels. A few are found in the sub-pleural exudate. They vary in length from 2 to 5·5μ, the average being about 3·5μ. At least half are curved.

SUMMARY.

Tubercle bacilli are distributed throughout the tissue in large numbers, but there are no tubercles and the tissue though congested, is not solidified. There is an abundant deposit of fibrin.

VIRUS.—H 25. A.T.
Animal Inoculated.—Calf 831.

Calf 831 was inoculated intravenously with an emulsion of the tissues of Calf 551, containing about 1,000,000,000 bacilli, and died 17 days afterwards.

Histological Changes.—The alveolar walls are thickened, owing partly to vascular engorgement and partly to an infiltration with leucocytes. A relatively small number of the alveoli are completely occluded, but the majority are still patent. There are no histological tubercles. There is no exudate of fibrin.

Distribution and Characters of Bacilli.—Tubercle bacilli are very numerous throughout the tissue. They vary in length from 2 to 5μ, the average being about 3·5μ.

SUMMARY.

The lungs are congested, but the amount of tissue change is very small in proportion to the large number of bacilli present.

VIRUS.—H 25. A.T.
Animal Inoculated.—Calf 859.

Calf 859 was inoculated intravenously with an emulsion of the tissues of Calf 831, containing about 2,000,000,000 bacilli, and died 19 days afterwards.

Histological Changes.—The tissue is in a condition of progressive and nearly complete solidification. There are many small foci which contain collections of nuclear débris, but there are no lesions which can be regarded as tubercles. No fibrin is demonstrable.

Distribution and Characters of Bacilli.—Tubercle bacilli are very numerous throughout the tissue, and form large groups in all the foci which, though they are not typical tubercles, contain collections of broken down cell nuclei. The bacilli vary in length, from 2 to 5μ, the average being about 3·5μ.

SUMMARY.

Pneumonic consolidation due to the presence of enormous numbers of tubercle bacilli. No fibrin.

VIRUS.—H. 29. M.F.
Animal Inoculated.—Calf 477.

Calf 477 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated with the original material. The dose was estimated to contain about 3,000,000,000 bacilli. Calf 477 was killed, when moribund, 34 days afterwards.

Histological Changes.—The tissue is occupied by large semi-confluent, caseous tracts. The groups of alveoli which are left between these areas are in many cases filled with a coagulated exudate. There is an abundant deposit of fibrin.

Distribution of Bacilli.—Bacilli are very numerous in the caseous areas.

SUMMARY.

Acute, progressive tuberculosis.

A CASE OF NON-TUBERCULAR PNEUMONIA.—Calf 615.

It is interesting to compare this case with those recorded above.

Calf 615 was not used for experiment. It died spontaneously. On post-mortem examination the lungs were found in a condition of breaking down broncho-pneumonia, and exhibited numerous yellow, softening foci not very unlike tubercles. Cocci were present in large numbers. The animal has been proved to be free from tuberculosis.

The following points of comparison with the tubercular lungs are worth recording.

General Characters.

(1) Consolidated areas more extensive than in the tubercular lungs.

(2) Areas of necrotic change more diffuse, and more marked absence of conservative change.

Details.

(1) *Alveolar Epithelium.*—Desquamation more extensive and disappearance much more rapid.

(2) *Oxyphil Leucocytes.*—The entire tissue, where not necrosed, is packed with multinuclear oxyphils. The enormous number of these cells present is one of the most marked features of difference from the tubercular lungs. In the latter specimens, as has been pointed out, their number was much greater in some cases than in others; but even where most abundant their number is very small in comparison with this lung.

(3) *Small Lymphocytes.*—Very scanty, in comparison with the multinuclear leucocytes.

(4) *Plasma Cells.*—Present, but much rarer than in the tubercular lungs. One or two are occasionally found within the consolidated areas. Sometimes well-stained groups are found at the periphery of bronchioles. A few of the plasma cells are in mitotic division.

(5) *Connective Tissue and Endothelial Cells.*—The disappearance of these cells is very rapid, and there is no evidence of any attempt at proliferation.

(6) *Elastic Tissue.*—There is some diminution in the amount of elastic tissue in the consolidated foci, but not so much as in the tubercular lungs. It must, of course, be remembered that the lesions in the tubercular specimens are of longer standing.

(7) *Fibrin.*—There is an abundant deposit of early fibrin, both in the blood vessels and in the rest of the tissue.

III. EXPERIMENTAL TUBERCULOSIS OF THE LIVER.*

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Heifer 30.

Heifer 30 was inoculated subcutaneously with an emulsion of the udder of Cow 40. This udder contained very few bacilli. Heifer 30 was killed 115 days after inoculation.

The liver contains large caseous tubercles which are completely surrounded by a very dense fibrous tissue zone, which is not completely avascular but appears to be extending peripherally. External to this zone is an area of infiltration, together with small patches of commencing necrosis. It is therefore evident that the tubercles are increasing in size. The liver capsule is thickened, and there is a cellular increase associated with the portal canals.

Tubercle bacilli are very rare, and are found only amongst the cells internal to the fibrous zone of the tubercles.

As in the lungs of this animal, described above, the tubercles are packed with multinuclear leucocytes. These cells occupy the interstices of the fibrous zone, and also form large masses internal to this area. They are also very abundant in the infiltration surrounding the tubercles and near to some of the portal canals. They all take a well marked oxyphil stain, and tend to have rather coarse granulation, especially when occurring amongst the fibrous tissue. There are not many lymphocytes. Plasma cells are numerous. They form a broad tract internal to the fibrous tissue zone; they infiltrate this zone; and they occur in large numbers in the external tissue. In the inner but non-caseous parts of the tubercles the liver cells are, to a large extent, replaced by endothelial cells.

Summary.

Chronic, slowly progressive, fibro-caseous tubercles, very poor in bacilli.

VIRUS.—B I.

Animal Inoculated.—Calf 102.

Calf 102 was inoculated subcutaneously with an emulsion from the tissues of Heifer 30, estimated to contain 116,000 bacilli, and was killed 150 days subsequently.

Large caseo-calcareous tubercles are present, bounded by a dense fibrous tissue zone. In addition to these, there are irregular tracts in the liver substance which show an infiltration with small cells, contain a good deal of adult fibrous tissue, and are partially caseous. There is also a fibrous tissue increase, and a small-celled infiltration in the portal canals, some of which contain definite tubercles. Giant cells are numerous in all three situations.

Four specimens have been carefully searched, but no tubercle bacilli have been found.

In the areas of dense leucocytic infiltration there is a good deal of coagulated material which takes a fibrin stain, but there are no delicate fibrin fibrils.

In all the lesions there is a dense infiltration of oxyphil multinuclear leucocytes. They all appear to be of the type usually described as finely granular, but the granulation of many of those found in the outer zones of the large circumscribed tubercles is distinctly coarse. Small lymphocytes are most numerous in the earlier and more irregular areas of infiltration and in the portal canals. Plasma cells are present in all the lesions. In the older lesions, the epithelioid type of cell is not a prominent feature and the proliferation of endothelial cells appears to have ceased. In the earlier lesions the endothelial proliferation appears to have expended itself in giant cell formation, and then to have been suppressed by the leucocytic infiltration. Another feature of the earlier lesions is the proliferation of the normal stellate cells associated with the venous capillaries of the liver.

Summary.

Chronic, caseo-calcareous tubercles, with much fibrous tissue increase. No bacilli found.

VIRUS.—B I.

Animal Inoculated.—Calf 126.

Calf 126 was inoculated subcutaneously with an emulsion of tissue from Calf 102, estimated to contain 110,000 bacilli, and was killed, when very ill, forty-five days afterwards.

The portions of liver tissue examined contain circular tubercles with a well-defined fibrous tissue outline, which vary in size from the area of the microscopic field (No. 4 eyepiece; 16 mm. objective) to about one-twentieth of this diameter. The largest are caseous with a central nodule of early calcification. Many of the nodules are close to and partially involve the portal canals. Occasionally a giant cell is found isolated amongst apparently normal liver parenchyma. The capsule is thickened, and in some places is partially separated from the liver substance by tissue oedematous in appearance and poor in cells.

The oldest lesions are the richest in bacilli. In one of the caseo-calcareous nodules examined tubercle bacilli are scattered very plentifully amongst the fine particles of calcareous matter; in the caseous zone surrounding this deposit their number is somewhat less; and in the outer cellular zone bacilli are few. In the smaller lesions and in the isolated giant cells, bacilli are only found with difficulty. None have been found in the areas of infiltration beneath the capsule.

No fibrin is satisfactorily demonstrable, although the oldest lesions contain a good deal of black staining deposit.

Only a few leucocytes are associated with the lesions. The other cellular constituents of the tubercles are of the usual type—a groundwork of endothelial cells, interspersed with small lymphocytes, external to these a

* In recording my observations I have maintained, as far as possible, the following order of description: (1) General low-power view. (2) Distribution of bacilli. (3) Presence or absence of fibrin in cases where it appeared at all likely that fibrin might be found. (4) Vascularity, when abnormalities are found and are related to the tubercular areas. (5) The distribution of leucocytes, lymphocytes, and plasma cells. (6) Any feature found noteworthy concerning the endothelial or the parenchymatous cells. (7) The presence or absence of fibrous tissue changes.

well-marked belt of plasma cells, and, separating the tubercles from the normal tissue, a fibrous zone. Plasma cells are not confined to the tubercular areas, but are conspicuous in the interstitial tissue surrounding nearly all the larger veins, particularly those contained within portal canals.

The subcapsular infiltration contains some plasma cells and empty capillary channels.

SUMMARY.

Chronic, fibro-caseous tubercles, with some calcification. Bacilli plentiful in some of the calcareous areas, less frequent in the caseous material, scanty in the outer cellular zone and within giant cells.

VIRUS.—B I.

Animal Inoculated.—Calf 132.

Calf 132 was inoculated subcutaneously with an emulsion, from organs of Monkey 62, estimated to contain about 1,000,000 bacilli. Monkey 62 had been fed with the milk of Cow 74. Calf 132 died 35 days after inoculation.

Numerous small tubercles, generally circular in outline, are distributed throughout the gland. Caseation is very advanced in most of them, and even in the smallest the central part is generally necrotic. The early onset of necrosis is a characteristic feature of the lesions found in this liver. Several of the lesions are adjacent to, and some of them actually involve, portal canals. In association with this fact it is interesting to note that Dr. Griffith found tubercles beneath the mucous membrane of the gall bladder. Apart from definitely tubercular changes, there is an increase of the interstitial elements in most of the portal canals.

Bacilli are found in all the tubercles, but not in large numbers. They only occur in the portal canals where there are definite signs of cellular degeneration. None have been found in other situations.

Fibrin is found in the tubercular lesions, and also in many of the blood vessels.

Demonstrable oxyphil leucocytes are infrequent in the tubercles, a fact which can hardly be due to *post-mortem* change, as these cells retain their staining properties in the veins and capillaries. The tubercles, where not necrotic, contain many small lymphocytes but only a few plasma cells. Surrounding most of the veins there are some plasma cells, and within Glisson's capsules these cells are numerous. The tubercles show very little endothelial or fibrous tissue increase.

SUMMARY.

Acute disseminated tuberculosis, with rapid necrotic change and evidence that the tissue resistance has been almost completely overcome. Fibrin present. Bacilli found in all the lesions, but not in large numbers.

VIRUS.—B II.

Animal Inoculated.—Calf 114.

Calf 114 was inoculated subcutaneously with an emulsion of tissue from Calf 22, estimated to contain something over 1,000 bacilli, and was killed 161 days subsequently.

Specimens have been prepared from two portions of the liver, each including a portion of the capsule.

In the first specimen there is a general thickening of the capsule, and at one point within it there is a projecting tubercle measuring about twice the diameter of the microscopic field (low power). The tubercle is surrounded by fibrous tissue, and except on its inner aspect, where this tissue slightly indents the liver parenchyma, is external to the level of the liver substance, being connected with it by a broad, fibro-vascular base. It is centrally caseous, with indications of commencing calcification, contains giant cells, and is identical in general appearance with the ordinary circumscribed tubercles found in the substance of many other livers which have been examined. In the interior of the gland the greater portion of the tissue is normal, but a giant cell is occasionally found and is generally associated with a small patch of infiltration. Some of these lesions occur within the lobules, and others at their periphery.

In the second piece of tissue examined there is a wedge-shaped area passing inwards from the capsule and penetrating some distance into the liver substance. The base of the wedge forms a projection above the general level of the capsule, and is occupied by œdematous tissue with no histological appearance of tuberculosis. The rest of the wedge, from its marginal infiltration, central caseation, and giant cells, has the general appearance of an ordinary tubercle. As in the previous specimen, there is a general thickening of the liver capsule, and a few giant cells are found in the substance of the gland.

In the interior of the projecting tubercle first described, tubercle bacilli are plentiful. Most of them are found in the caseo-calcareous area, but the outer zone of epithelioid and giant cells also contains several. No bacilli are found external to this zone, nor do they occur anywhere in the loose connective tissue of the thickened capsule. The wedge-shaped tubercular focus contains a few bacilli in its interior, but none in its capsular margin, nor do bacilli occur in the adjoining parts of the liver capsule. A few bacilli are found in some of the small foci noted in the substance of the gland.

With the exception of some old coagulated deposit found in the caseous part of the projecting tubercle, there is no evidence of fibrin in any part of the tissue.

The histology of the tubercle which forms a projection within the capsule is of special interest, because it is free from all association with the liver parenchyma. Oxyphil leucocytes are present in it in small numbers. Small lymphocytes are much more numerous. Plasma cells are abundant and form a well-marked peripheral zone; many of them are in process of transition into fibroblasts. The chief cellular constituent of the tubercle consists of endothelial cells. These can be traced as a proliferation from the walls of the numerous marginal capillaries. As the cells pass inwards they lose their tendency to grow in parallel columns, their protoplasm becomes swollen, and they form a typical epithelioid zone. Giant cells are also formed from them. The vascular supply of the tubercle extends close up to the area of caseation. The fibrous tissue boundary of the tubercle is well defined in van Gieson specimens. The close resemblance which this tubercle presents to ordinary chronic tubercles, found within the hepatic lobules, supports the view that in the formation of the latter the parenchymatous cells do not play a permanent part.

In the wedge-shaped tubercle the infiltration is mainly composed of small lymphocytes and ordinary fibroblasts. There is a noticeable absence of plasma cells. Amongst this infiltration are groups of partially broken-down liver cells. Oxyphil leucocytes are found chiefly in the last situation.

The histology of this liver has some bearing on the development of the nodular outgrowths which are regarded as typical of bovine tuberculosis. In passing from the adjacent liver capsule, which rests upon normal parenchyma, to the projecting tubercle above described, the following stages of transition can be observed:—(1) The capsule is thickened and œdematous; the connective tissue fibres are swollen. (2) A few extraneous cells, lymphocytes, and occasionally plasma cells, make

their appearance. (3) The capsule is penetrated on its inner aspect by endothelial cells; these can sometimes be traced from the adjacent blood capillaries in the liver substance. (4) Numerous capillary channels containing red corpuscles are seen. (5) The outermost zone of the tubercle, consisting mainly of fibroblasts and plasma cells, is now reached. The epithelium on the peritoneal aspect of the capsule takes no part in these changes. Tubercle bacilli are not found until the interior of the tubercle is reached.

It may be useful to associate these observations with other records I have made of outgrowths found on the pleural or peritoneal surface of bovine lungs and livers. In the first stage of development these outgrowths consist merely of œdematous or myxomatous tissue. The second stage in the process is vascularisation. In the third stage they contain tubercle bacilli, and are histologically tubercular. In the first two stages I have not found them associated with tubercle bacilli, nor do they exhibit any histological evidence of tuberculosis.

So far as my observations extend, I am inclined to the view that these outgrowths owe their origin, not to the presence of a specifically "bovine" bacillus, but to an idiosyncracy of bovine tissues. If tubercle bacilli chance to get into the circulation, they may find in these newly vascularised situations a place of least resistance, and thereupon lead to a luxuriant growth of tubercles. This tubercular growth, though typical of bovine tissues, is not necessarily an indication that the bacilli present are of bovine origin.

SUMMARY.

Caseo-calcareous tubercles in the substances of the liver and mushroom growths projecting from the capsule. Bacilli fairly plentiful in the interior of the tubercles.

VIRUS.—B V.

Animal Inoculated.—Calf 144.

Calf 144 was inoculated subcutaneously with an emulsion of original material estimated to contain 4,500,000 bacilli and was killed 78 days afterwards.

Tubercular lesions of large size occur irregularly, and are characterised by the presence of an unusually large amount of fibrous tissue which not only surrounds caseous foci, but ramifies into the adjacent tissue. Giant cells are abundant, both within these lesions and amongst the liver parenchyma which is external to them. The interlobular tissue shows in many places an increase of fibrous elements, and an infiltration with small cells.

Several sections have been searched for tubercle bacilli, but none have been found.

The interior of the tubercles is filled with the nuclear remains of multinuclear leucocytes, whilst at their periphery these cells are also very numerous, and retain a good oxyphil reaction. Oxyphil leucocytes are also very numerous in the patches of infiltration outside circumscribed tubercles. The other constituents of the tubercles are small lymphocytes, endothelial cells in a state of partial disintegration, and plasma cells. Cells of the last type are especially numerous, and invariably play the chief part in the fibrous tissue formation.

SUMMARY.

Large caseous tubercles surrounded by a very dense fibrous zone which is extending peripherally and ramifying into the adjacent tissue. Extensive leucocytic infiltration. No bacilli found.

VIRUS.—B IX.

Animal Fed.—Calf 380.

Calf 380 was fed with 1 mg. of culture of B IX. and was killed 140 days afterwards.

Large tubercles are present. They are partially caseous, contain many giant cells and are infiltrated with cells of which the greater number are lymphocytes. They exhibit a peripheral zone of fibroblasts.

Many much smaller foci are also present. In some of these, giant cells occur.

Tubercle bacilli are very rare. Occasionally one is found within a giant cell.

SUMMARY.

Caseous tubercles, of a chronic type, are numerous. They contain very few bacilli.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 153.

Calf 153 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated with the tissues of Heifer 13. Calf 153 was killed, when very ill, thirty-nine days after inoculation.

Small irregular tubercles with only slight fibrous tissue demarcation are distributed in large numbers throughout the liver. Some of them involve the portal canals, others border upon hepatic veins, and others again have apparently originated amongst the hepatic parenchyma. Most of them are in early stages of caseation. Giant cells are not found.

Tubercle bacilli, for the most part long and curved, are found in the majority of the lesions, but are not at all numerous.

The tubercles contain some blood capillaries and extravasated red corpuscles, but are not markedly vascular.

Early fibrin is present in most of the lesions.

Oxyphil leucocytes, small lymphocytes, and ordinary fibroblasts are all present in large numbers in the tubercles. Plasma cells are infrequent, and do not form a definite boundary zone. There is not much evidence of a proliferation of cells which can be definitely recognised as endothelial, nor are newly formed capillary channels noticeable.

SUMMARY.

Acute disseminated tuberculosis. Fibrin present. Bacilli not numerous.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 103.

Calf 103 was inoculated subcutaneously with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli. The animal was killed 130 days afterwards.

The specimen of liver contains many small foci which are characterised by an aggregation of finely granular oxyphil leucocytes, but do not exhibit any other noteworthy change. They are not caseous, do not contain giant cells, and have none of the characteristic structure of tubercles.

No tubercle bacilli have been found in these lesions.

Summary.

Small foci of doubtful nature are present. No tubercle bacilli have been found in them.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 105.

Calf 105 was inoculated subcutaneously with 48,000 bacilli contained in an emulsion of the tissues of Calf 5. Calf 105 was killed, when moribund, forty-seven days later.

The lesions in this liver resemble those in the liver of Calf 114, which was inoculated with B II. There are fibro-caseous tubercles which form flattened projections in the capsule and do not involve the liver parenchyma, subcapsular wedge-shaped tubercles, small areas of infiltration, and isolated giant cells.

Tubercle bacilli are not quite so numerous as in Calf 114, but their distribution is the same. None are found in the capsule except within definitely circumscribed tubercles.

Histologically both the projecting and the subcapsular tubercles resemble the projecting tubercle described in Calf 114; the only difference being that the endothelial proliferation is somewhat less active.

Summary.

Closely comparable, as regards both the internal tubercles and the mushroom growths on the capsule, to the lesions produced in Calf 114 by a bovine virus.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 109.

Calf 109 received an intraperitoneal inoculation with an emulsion from the tissues of Calf 5 estimated to contain 48,000 bacilli, and died 40 days afterwards.

Beneath the capsule there is a large caseating tubercle. The zone of cells which separates this tubercle from the liver parenchyma is not well defined, but appears to be spreading into the liver substance. In the interior of the liver there are also numerous small miliary tubercles, most of which are in an early stage of caseation, though some have not commenced to caseate.

Bacilli are fairly numerous in the tubercle first mentioned, but are scanty in the smaller lesions.

Summary.

Disseminated tuberculosis. Bacilli are scanty in the smaller and earlier lesions, but more numerous in the older lesions.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 141.

Calf 141 was inoculated subcutaneously with an emulsion of bacilli, estimated to number 250,000, from the tissues of Cow 73. Calf 141 was killed fifty-three days after inoculation.

At one part of the capsule is a flattened projection consisting of myxomatous tissue, and containing a few capillary vessels. In the substance of the gland there are several caseous tubercles, which are isolated and more or less circular in outline, but have no fibrous tissue demarcation. Frequently, but not invariably, they are situated close to portal or hepatic veins.

The subcapsular projection contains no tubercle bacilli. In the tubercles contained within the liver substance bacilli are fairly plentiful.

There is no fibrin in the subcapsular projection, nor is there any recent fibrin in any part of the liver substance.

The characteristic of the tubercles is caseation, with very little compensatory formation of granulation tissue. In association with this condition it is important to note that plasma cells are rare, and those which are present stain badly.

Summary.

Caseous tubercles, not circumscribed by fibrous tissue. Bacilli fairly plentiful. No fibrin demonstrable.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 305.

Calf 305 was inoculated intravenously with an emulsion, estimated to contain about 730 million bacilli, of the organs of guinea-pigs, which had been inoculated from Calf 177, and was killed, when very ill, twenty-six days after inoculation.

There is a diffuse, cellular infiltration which is uniformly distributed. It is for the most part interstitial; it is associated with the portal canals, the vessels at the margins of the lobules, some of the larger hepatic veins, and the vessels beneath the capsule. The liver cells are also involved but, in a low-power view, to a smaller extent. The lesions involving the liver cells are generally circular in outline, and exhibit cell fusions, occasionally giant cells, and commencing necrosis.

Tubercle bacilli are present in all the lesions, and are generally long and beaded. Though nowhere scanty in numbers, they are least abundant in those lesions which do not involve the liver cells. Many bacilli appear definitely intracellular, and in the small circular lesions which involve the liver cells many of the cells containing bacilli can be recognised as belonging to the liver parenchyma. Some of these lesions are extremely small, consisting only of a few liver cells containing bacilli, and a still smaller number of adventitious cells. Tubercle bacilli are also frequently found within, or closely attached to, liver cells which are otherwise normal in their appearance, and are situated in a normal environment. Bacilli also occur, but much less frequently, within venous capillaries. Only one has been found in a large vessel; it was lying free, and may have been accidentally carried into that position in the preparation of the section.

Early fibrin is present in the tubercular foci.

Oxyphil leucocytes are found in fairly large numbers in all the lesions; they occur even in the earliest, where the liver cells show any signs of breaking down. In the blood-vessels and capillaries there is evidence of a high degree of leucocytosis. Small lymphocytes, possessing no recognisable protoplasm in Pappenheim specimens, are present, but are not, on the whole, as numerous as the multinuclear leucocytes. Plasma cells are abundant, especially around blood-vessels and throughout the inter-lobular interstitial tissue. Amongst the small intralobular tubercles they are also found, but are less numerous here than in the interstitial tubercular areas. Isolated plasma cells are also occasionally found amongst liver cells where there is no other histological indication of pathological change. Occasionally a cell with the staining reaction of a plasma cell is found within a blood-vessel; these may be ordinary large lymphocytes.

The above are the only changes observed in the small intralobular foci. In the interstitial lesions there is also a proliferation both of endothelial cells and of ordinary fibroblasts.

SUMMARY.

Early tubercles. Fibrin present. The amount of tissue destruction is very small, relatively to the large number of bacilli present and their wide distribution.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 361.

Calf 361 was inoculated intravenously with an emulsion, estimated to contain 41,500,000 bacilli, from the tissues of Calf 275, and was killed 76 days afterwards.

The liver contains many small tubercles. They are all of the quiescent type and have broad demarcation areas consisting, to a large extent, of fibroblasts and lymphocytes. They contain many giant cells, and the larger of the tubercles are completely caseous in the centre.

Tubercle bacilli are very rare.

SUMMARY.

Disseminated tubercles of a retrogressive type.

VIRUS.—H 9. C.T.

Animal Inoculated.—Calf 185.

Calf 185 was inoculated subcutaneously with ·75 c.c. of wet bacilli grown on glycerinated broth. The culture was $4\frac{1}{2}$ months old. The animal was killed forty days afterwards.

In the specimens examined a few small tubercles are found, occurring generally either immediately beneath or near to the capsule. In the liver substance are a few very small aggregations of small round cells, and here and there a single large giant cell occurs, which, with the exception of a few smaller cells in contact with it, appears to be situated amongst normal liver substance. There appears to be a slight inflammation extending from some of the areas enclosed within Glisson's capsule. These are all the abnormalities noted with a low power.

Several specimens have been searched for tubercle bacilli, but only one or two bacilli have been found. They occur within large giant cells.

There is no indication of fibrin in the tubercular areas. The specimens do not stain readily by Pappenheim's method; no plasma cells have been demonstrated. Multinuclear leucocytes are abundant in the tubercles, and in these situations are filled with rather small but very definite granules; they have no particular relationship to the giant cells found within the same areas. Multinuclear leucocytes also occur in fairly large numbers throughout the liver substance; they sometimes form small aggregations, but for the most part occur singly, amongst normal liver cells; many of them are identical with those found in the tubercles, others are rather less definitely granular. There is no constant association between the isolated giant cells found in the liver substance and multinuclear leucocytes; in the neighbourhood of some of these giant cells there is a complete absence of multinuclear leucocytes. In what I have described above "as small aggregations of small round cells" within the liver substance, oxyphil leucocytes are present. Lymphocytes of the ordinary type, with a round, deeply staining nucleus and relatively little protoplasm, are abundant in the tubercles, very frequent throughout the liver substance, and account for more than half the cells in the "aggregations of small round cells." In the centre of the definite tubercles are many cells which, in the shape and character of their nuclei and in the granular appearance of their protoplasm, resemble the parenchymatous cells of the liver. Breaking down liver cells are also to be found within the small aggregations of small round cells. An examination of the nuclei of the giant cells found in these sections does not justify a definite opinion as to their origin. There is not much evidence in the tubercle of newly formed cells of definitely endothelial type.

SUMMARY.

Small, retrogressive lesions, containing very few bacilli.

VIRUS.—H 10. B.S.

Animal Inoculated.—Calf 191.

Calf 191 was inocultated subcutaneously with an emulsion from the tissues of Calf 35 estimated to contain about 2,500,000 bacilli, and was killed, when moribund, 60 days afterwards.

The liver contains many large tubercles which are in an advanced stage of caseation. There is a layer of lymphocytes and leucocytes at the margin of these tubercles, but no fibrous zone. Many much smaller tubercles, which have not for the most part, advanced to caseation, are also present.

Bacilli are scanty in the small tubercles; they are more plentiful, though not very numerous, in the larger. They occur more frequently in the peripheral zone than in the caseous areas.

SUMMARY.

Progressive tuberculosis.

VIRUS.—H 10. B.S.

Animal Inoculated.—Heifer 231.

Heifer 231 was inoculated subcutaneously with an emulsion of the tissues of Calf 113 containing 1,800,000 bacilli, and was killed 109 days afterwards.

The portion of the liver examined contains a flattened mushroom-like outgrowth attached to the capsule by a narrow pedicle. This outgrowth contains several giant cells and a few patches of early caseation. The rest of the liver capsule is uniformly thickened and œdematous. Beneath it there are a few small tubercular foci containing giant cells. A few still smaller foci are found more in the interior of the liver substance.

In a caseous area of the mushroom-like outgrowth one tubercle bacillus has been found. None have been seen elsewhere.

No fibrin is present.

The ground substance of the flattened-out growth consists of a widely open network of branching connective tissue fibrils. This is permeated by large number of capillary channels, and also contains a few arteries and veins. Many capillaries are seen in process of formation. Endothelial cells are also found growing irregularly; sometimes they form groups of cells partially fused together, and commencing to break down, and in many places they have given rise to typical giant cells. Oxyphil leucocytes are scattered about in moderately large numbers; they nowhere form definite aggregations, nor are they particularly numerous in the neighbourhood of the giant cells or breaking-down endothelial cells. Small lymphocytes are more numerous than any other type of cells; they are present everywhere, and in some parts of the outgrowth are packed as closely as in a lymphatic gland. Plasma cells are also abundant, and are disseminated throughout the outgrowth. They are not associated with the giant cells, nor are any found within blood-vessels or capillaries. Most of them have a round or polygonal rather than a compressed outline; they do not form the close aggregations or parallel columns which are always to be found when these cells form the demarcation of more advanced tubercular foci. Both in their shape and their distribution they bear a close resemblance to the large lymphocytes in a normal lymphatic gland.

The tubercular lesions found in other parts of the liver are surrounded with a zone of lymphocytes and generally contain giant cells. They are partially caseous, but contain very few bacilli and appear to be retrogressive.

SUMMARY.

A typical mushroom growth produced by a human virus.

VIRUS.—H 12. H.N.

Animal Inoculated.—Calf 319.

Calf 319 was inoculated intravenously with an emulsion, estimated to contain over 100 million bacilli, of the organs of guinea-pigs which had been inoculated from Calf 189, and was killed, when moribund, 57 days afterwards.

No tubercular lesions are visible with a low power, but there is a slight increase of the cellular elements of the interstitial tissue surrounding the large veins. The capsule is somewhat thickened and more vascular than normal, but exhibits no projections on the surface.

Tubercle bacilli are generally distributed throughout the interlobular connective tissue. They are also particularly numerous throughout the entire length of the portion of the capsule examined. This is an unusual feature. In both these situations many of the bacilli are intracellular. Small groups of bacilli are also found within liver cells. Some of these cells exhibit large vacuoles, but the majority of them appear normal; there is very little tendency amongst them to cell fusions, and there are no giant cells. There are not, as a rule, any leucocytes or other adventitious cells in proximity to the parenchymatous cells which contain bacilli. With the exception of a few places where the capillary walls are not intact, no bacilli are found within blood capillaries. They are also absent from the larger blood-vessels.

No fibrin is present.

Oxyphil leucocytes are rare in the interstitial tissue, and are not present in the capsule. They are not particularly numerous in the blood capillaries, nor do they form aggregations in any part of the tissue. A few plasma cells are found in the interlobular tissue, and similar cells are occasionally seen within venous capillaries. The chief increase in the interstitial tissue is due to ordinary fibroblasts, bearing no resemblance to plasma cells.

The main feature of the tissue is the extremely small amount of tissue reaction to the bacilli present.

SUMMARY.

No definite histological tubercles. No fibrin. Bacilli very numerous; relatively to their numbers very little tissue reaction.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 301.

Calf 301 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated from Calf 129. Calf 301 was killed, when very ill, thirty-three days after inoculation.

Many small isolated tubercles are present both beneath the capsule and more deeply in the substance of the gland. The foci beneath the capsule are the largest. All the tubercles involve the liver parenchyma. Many of them border upon large veins, but the portal canals are not, as a rule, involved. The tubercles have not begun to caseate or are only slightly caseous, but in their centres cell fusions and giant cells are found.

In Ziehl-Neelsen specimens, many of the cell groups last mentioned exhibit under a low power a red area in their centre. These areas are clusters of tubercle bacilli. Throughout the tubercles bacilli are abundant, and are nearly all intracellular. It is the exception for the bacilli to occur singly; each cell or cell group in which they are present generally contains one or more small clumps. Apart from the tubercles, bacilli are not found in the rest of the liver substance, with the exception of a few of the veins. Two sections have been completely searched, and in four hepatic veins bacilli have been found adherent to or within the endothelial cells lining the lumen of the vessel; in two other large vessels bacilli have been found lying free in the lumen.

A little fibrin is frequently found in association with some of the larger clusters of bacilli.

Oxyphil, finely granular leucocytes are present in all the lesions, but are not the predominating feature in any of them. They are also present in greater than normal numbers throughout the blood capillaries. Small lymphocytes are also found in all the lesions, and occur in much larger numbers than oxyphil leucocytes. Round the larger tubercles there is a well-marked zone of plasma cells, many of which are passing into fibroblasts. In the smallest foci only a few plasma cells have made their appearance. In the interlobular interstitial tissue plasma cells are only found in situations close to tubercular foci. The characteristic feature of all but the smallest tubercles is the replacement of the liver parenchyma by newly formed endothelial cells. These cells form the chief constituent of the tubercles, and can often be traced as a proliferation from the marginal capillary endothelium. They are probably the source of most of the giant cells and cell fusions.

There is a fairly definite fibrous tissue zone surrounding some of the tubercles.

Summary.

Numerous miliary tubercles, often containing giant cells and showing more evidence of tissue resistance than is usual in very acute cases. Bacilli are abundant; they are very frequently intracellular. Fibrin is present.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 325.

Calf 325 was inoculated subcutaneously with an emulsion from the tissue of Calf 301 containing, approximately, 500,000,000 bacilli. Calf 325 was killed, when moribund, 24 days afterwards.

Small miliary tubercles are present in large numbers throughout the substances of the liver. These tubercles are slightly caseous; they are invaded by leucocytes and contain some lymphocytes at their periphery. They have no fibrous boundary. Occasionally an imperfectly formed giant cell is present. There is an abundant deposit of fibrin.

Tubercle bacilli are numerous.

Summary.

Acute miliary tuberculosis.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 121.

Calf 121 was inoculated subcutaneously with an emulsion of the original material estimated to contain over 360,000,000 bacilli. The animal was killed, when ill, 36 days afterwards.

The liver contains numerous small miliary tubercles, which are caseating, obviously progressive, and show no sign of conservative change.

The tubercles contain numerous bacilli.

Summary.

Progressive miliary tuberculosis.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 125.

Calf 125 was inoculated subcutaneously with the same dose of the same material as Calf 121, and was killed 23 days afterwards.

The liver contains numerous miliary tubercles which are caseous and, though not definitely circumscribed, contain at their periphery numerous fibroblasts as well as lymphocytes.

The tubercles contain bacilli, but in smaller numbers than those in the liver of Calf 121.

Summary.

Miliary tuberculosis. The lesions are somewhat less acute in type than those found in the liver of Calf 121.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 197.

Heifer 197 was inoculated subcutaneously with an emulsion of the tissues of Calf 121 containing, approximately, 85,500,000 bacilli. Heifer 197 was killed, when very ill, fifty-one days afterwards.

The liver contains large tubercles, which are in an advanced stage of caseation, and are surrounded by dense walls of fibrous tissue.

The tubercles contain bacilli, but only in small numbers.

They are well supplied with blood capillaries, both in their outer zones and in their interior; even amongst the caseous material many intact blood channels filled with red corpuscles are found. In many places there are extravasations of red corpuscles.

The tubercles contain a large amount of fibrin. Near their periphery this fibrin has the appearance of recent formation; it is not, for the most part, associated with tubercle bacilli. No fibrin is found within intact blood-vessels.

Leucocytes are present within the tubercles in very large numbers, but the greater proportion of them have lost their oxyphil stain. Small lymphocytes are nearly as numerous as the multinuclear leucocytes. Plasma cells do not stain well, but some are demonstrable. The most marked feature of the periphery of the tubercles is the proliferation of endothelial cells and the formation of new blood capillaries.

On the whole, the general features of the lesions appear to indicate vigorous tissue resistance.

SUMMARY.

Large caseous tubercles, surrounded by a dense fibrous wall and not containing many bacilli. The tubercles are well supplied with blood capillaries. Fibrin is present.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 243.

Heifer 243 was inoculated subcutaneously with an emulsion of tissue from Heifer 197 containing about 4,500,000 bacilli. Heifer 243 was killed, when very ill, 61 days afterwards.

The lesions are of the same type as in Heifer 197. The only differences are that tubercle bacilli are slightly more numerous, multinuclear leucocytes are fewer, whilst plasma cells are much more numerous and stain well. As in Heifer 197, fibrin is found in very considerable amount.

SUMMARY.

Similar to Heifer 197.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 273.

Calf 273 was inoculated subcutaneously with an emulsion from the tissues of Calf 187 containing, approximately, 11,000,000 bacilli. Calf 273 was killed 89 days afterwards.

Beneath the capsule there is a tubercle which is surrounded by a broad fibrous zone. Its centre is caseous and contains some calcareous particles. In the substance of the liver there are several minute foci which can be recognised as tubercles but generally consist of no more than a giant cell and a small aggregation of lymphocytes and leucocytes.

A few bacilli are present in the caseo-calcareous tubercle, but none have been found elsewhere.

SUMMARY.

Isolated tubercles, of a retrogressive type. Bacilli rare.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 355.

Calf 355 was inoculated subcutaneously with an emulsion of the tissues of Calf 273 containing, approximately, 3,000,000 bacilli. Calf 355 was killed, when very ill, 43 days afterwards.

The specimen contains a caseous tubercle, which is partially bounded by fibroblasts, and very numerous smaller foci. The latter have not advanced to caseation, but generally consist of a few giant cells surrounded by a large number of lymphocytes and leucocytes and some plasma cells.

The larger tubercle contains several bacilli; in the smaller, bacilli are rarely met with.

SUMMARY.

Numerous tubercles of a chronic type, containing few bacilli.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 529.

Calf 529 was inoculated intravenously with an emulsion from the tissues of Calf 539 containing, approximately, 1,000,000,000 bacilli. Calf 529 died 13 days afterwards.

The tissue is riddled with pale semi-necrotic tracts. Many of these tracts surround, and appear to be spreading from the periphery of, hepatic veins; they are unlike

ordinary lesions due to the actual presence of tubercle bacilli, but are more suggestive of a necrotic change due to the extravasation of toxic material.

Lesions are also present which show an increase of the fibrous and endothelial elements and contain aggregations of leucocytes; these are more suggestive of tubercles. No fibrin is demonstrable.

Tubercle bacilli are found in the blood-vessels and in all parts of the tissue which appear histologically normal. In the areas which are histologically suggestive of tuberculosis they are numerous, but they are very scanty in the pale, necrotic tracts mentioned above; this difference in their distribution is noteworthy.

SUMMARY.

Diffuse necrosis suggestive of a toxic infection, also small lesions more resembling tubercular foci. In the latter bacilli are numerous; they are also found in the blood capillaries, but are rare in the areas of diffuse necrosis.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 555.

Calf 555 was inoculated subcutaneously with an emulsion from the tissues of Calf 553 containing, approximately, 150,000,000 bacilli. Calf 555 was killed when moribund 58 days afterwards.

Forming a flattened projection beneath the capsule there is a caseating tubercle which contains an abundant deposit of fibrin and is very rich in bacilli.

The rest of the specimen appears normal, with the exception of a few minute, early foci. In these, tubercle bacilli are present.

SUMMARY.

Caseous tubercles, rich in bacilli, are present, and further dissemination appears to be taking place.

VIRUS.—H 18. T.T.

Animal Inoculated.—Calf 165.

Calf 165 was inoculated subcutaneously with an emulsion, estimated to contain about 4,400,000 bacilli of the original material, and was killed 57 days afterwards.

The specimen contains two tubercles which are completely surrounded by fibrous tissue and contain many giant cells. They are not caseating, but still contain recognisable cells of the liver parenchyma. They are permeated by some newly formed blood capillaries.

Bacilli are present in the tubercles, but are very rare.

SUMMARY.

Isolated tubercles, evidently non-progressive, are present.

VIRUS.—H 19. S.W.

Animal Inoculated.—Calf 159.

Calf 159 was inoculated subcutaneously with an emulsion estimated to contain over 1,500,000 bacilli of the original material, and was killed 50 days afterwards.

The specimen contains two large tubercles which are partially surrounded by fibrous tissue. One of them is in an advanced stage of caseation, the other is only slightly caseous and contains many lymphocytes and leucocytes, together with partially obliterated fixed tissue cells.

Both in the centres and at the periphery of the tubercles bacilli are present in small numbers.

SUMMARY.

Large tubercles of a chronic type are present.

VIRUS.—H 19. S.W.

Animal Inoculated.—Calf 271.

Calf 271 was inoculated subcutaneously with an emulsion from the tissues of Calf 179 containing, approximately, 16,000,000 bacilli. Calf 271 was killed, when very ill, 36 days afterwards.

The liver contains numerous small, semi-necrotic foci, in which the cellular remains do not react well to protoplasmic stains. The foci are not circumscribed by fibrous tissue.

In these foci tubercle bacilli are abundant. None have been found elsewhere, with the exception of one which is present within a liver cell bordering upon a hepatic vein.

The tubercular foci contain a fibrinous exudate, and early fibrin is found in some of the blood-vessels.

The cells in the tubercular lesions stain very badly, but remains of leucocytes, lymphocytes, and plasma cells can be made out. There is no indication of active increase of any type of cell.

SUMMARY.

Acute miliary tuberculosis. Bacilli abundant.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 399.

Calf 399 was inoculated intravenously with an emulsion of the lesions of Guinea-pig 200 containing over 700,000,000 bacilli, which was one of a series inoculated from Calf 291. Calf 399 was killed when very ill, 34 days afterwards.

Throughout the substance of the liver there are large numbers of minute foci which show very little tissue destruction, but generally contain a small collection of leucocytes and lymphocytes. In some of these foci there is an increase of cells of endothelial type. In a few only of the foci there is to be found a small amount of fibrinous deposit.

Tubercle bacilli are numerous in all the lesions and also occur in the rest of the tissue. Some have been found within blood-vessels, attached to mononuclear cells.

SUMMARY.

Bacilli are numerous and widely distributed. Some of the lesions in which they occur have the structure of early tubercles.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 749.

Calf 749 was inoculated intravenously with 46 mg. of culture isolated from Calf 293, and was killed when very ill, 24 days afterwards.

The liver shows an infiltration of small lymphocytes and leucocytes, which are often aggregated into small foci. These foci are not sufficiently definite in structure to be described as tubercles. No fibrin is demonstrable.

Bacilli are numerous in these foci and are also found scattered over the rest of the tissue.

SUMMARY.

Bacilli are distributed throughout the tissue. There are many small lesions, but these cannot be described as definite tubercles.

VIRUS.—H 23. J.P.

Animal Inoculated.—Calf 365.

Calf 365 was inoculated subcutaneously with an emulsion of original material estimated to contain about 100,000,000 bacilli. The animal was killed 61 days afterwards.

Tubercles are present, but they are smaller and less advanced than those found in the lungs of this animal, and much less numerous. The majority of them are interlobular. Giant cells are found in some of them.

No tubercle bacilli have been found.

The tubercles contain small groups of oxyphil leucocytes, a much larger admixture of lymphocytes and fibroblasts, and a few plasma cells.

SUMMARY.

Small, retrogressive tubercles, in which no bacilli have been found.

VIRUS.—H 23. J.P.

Animal Inoculated.—Calf 441.

Calf 441 was inoculated intravenously with an emulsion of the tissues of Calf 345, containing over 13,000,000 bacilli. Calf 441 was killed 102 days afterwards. It was found at the post-mortem examination that part of the inoculated material had lodged in the wall of the vein and amongst the surrounding tissue.

The specimen contains two tubercles, each about the diameter of the microscopic field (low power). The tubercles are not caseous, but contain a few giant cells. They are overrun with small lymphocytes. Plasma cells are also fairly numerous at their margins. Leucocytes are much less numerous than lymphocytes. In many other situations, chiefly interlobular, there is an interstitial infiltration, which consists chiefly of lymphocytes and plasma cells.

No tubercle bacilli have been found.

SUMMARY.

Retrogressive tuberculosis.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 551.

Calf 551 (a bull calf ten months old) was inoculated intravenously with 100 mg. of culture isolated from Calf 417, and died 21 days afterwards.

There are numerous early lesions, each consisting of a small focus where the parenchymatous cells are partially obliterated and are invaded by a group of leucocytes and lymphocytes. Fibrin is present in a few, but not in the majority of the lesions.

Bacilli are present in the lesions (a few in each), and also occur within the blood-vessels and in other situations where there are no definite histological changes.

SUMMARY.

Bacilli are distributed throughout the tissue, but do not form dense groups. Small foci are numerous which show very little tissue destruction, and have not advanced to the formation of typical tubercles.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 831.

Calf 831 was inoculated intravenously with an emulsion of the tissue of Calf 551 containing about 1,000,000,000 bacilli, and died 17 days afterwards.

Throughout the tissue there are numerous small areas in which the parenchymatous cells are partially obliterated. These areas are invaded with leucocytes and lymphocytes, and in some of them there appears to be an increase of the endothelial element. Many of the foci contain a fairly abundant deposit of fibrin. The amount of tissue destruction is slight.

Tubercle bacilli are present in all the lesions and are plentiful in some of them.

SUMMARY.

General dissemination of bacilli, with numerous small lesions.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 859.

Calf 859 was inoculated intravenously with an emulsion of the tissues of Calf 831, containing about 2,000,000,000 bacilli, and died 19 days afterwards.

The tissue shows very few lesions and no definite tubercles.

Tubercle bacilli are irregularly distributed, but are not numerous. Those present are generally long and curved.

SUMMARY.

Tubercle bacilli are present, but not in large numbers. They have produced very little tissue change.

VIRUS.—H 29. M.F.

Animal Inoculated.—Calf 477.

Calf 477 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs, which had been inoculated with the original material. The dose was estimated to contain about 3,000,000,000 bacilli. Calf 477 was killed, when moribund, 34 days afterwards.

The tissue contains numerous miliary tubercles which vary in size and are in various stages of caseation. The tubercles are not surrounded by fibroblasts. Most of them contain an abundant deposit of fibrin.

Tubercle bacilli are numerous in all the tubercles.

SUMMARY.

Progressive miliary tuberculosis.

IV. EXPERIMENTAL TUBERCULOSIS OF THE KIDNEYS.

A. LESIONS NOT FOUND ASSOCIATED WITH TUBERCLE BACILLI.

The investigation of bovine kidneys has been complicated by the presence of lesions in which, after repeated search, no tubercle bacilli could be found, and which are not histologically typical of tuberculosis. Some of these lesions are described first.

It is quite possible that in some other portion of the kidneys in which these lesions occurred a genuine tubercle may have been present, and it is also possible that the lesions described may have been due to a general condition of ill-health induced by the tubercular infection. In the subsequent description of definitely tubercular kidneys it will be seen that tubercular foci may occur side by side with lesions similar to the non-specific lesions which are first described.

VIRUS.—B I.

Animal Inoculated.—Calf 48.

Calf 48 was inoculated subcutaneously with an emulsion of the tissues of Monkey 18, and was killed 185 days afterwards.

In the cortex there are small patches of variable outline consisting chiefly of cells with small round nuclei. Some of these areas are close to large capsular veins; many of them border upon, but do not involve, glomeruli. In the largest areas of infiltration the renal tubules have been surrounded and partially disintegrated, and often present under these circumstances an appearance suggestive of giant cells. More in the interior of the gland the patches of infiltration are most frequently met with near to the large veins. A streaky infiltration is also noticeable between some of the medullary tubules which are cut in longitudinal section.

The cells with small round nuclei have the morphological characters and staining reactions of small lymphocytes. There are very few large lymphocytes. No oxyphil leucocytes are demonstrable. Amongst the small lymphocytes are also cells which, from the character of their nuclei, appear to be young connective tissue corpuscles; van Gieson specimens show that the increase of adult fibrous tissue is only slight.

VIRUS.—B I.

Animal Inoculated.—Calf 126.

Calf 126 was inoculated subcutaneously with an emulsion of the tissues of Calf 102, estimated to contain 110,000 bacilli, and was killed, when very ill, forty-five days afterwards.

Passing inwards from the capsule are a few small, wedge-shaped areas in which the interstitial tissue between the renal tubules and glomeruli is occupied by an infiltration which is not dense and has not obliterated the renal parenchyma. In this infiltration small lymphocytes, though fairly numerous, do not form dense aggregations; their numbers are greatest immediately beneath the capsule and close to glomeruli. Cells with the staining reactions of plasma cells are numerous and conspicuous throughout the infiltrated area; many of them are passing into fibroblasts. Finely granular oxyphil leucocytes, with a strong oxyphil reaction are fairly numerous; their number is greatest where the small lymphocytes are most numerous. In the interstitial stroma are found fine irregular fibrils, which stain red by van Gieson's method, but there is no definite increase of adult fibrous tissue.

Apart from the areas described, the only abnormality noticed in the tissue is the presence of small groups of plasma cells near to the outer coats of some of the larger blood-vessels which are situated between the cortical and the medullary parts of the gland.

VIRUS.—B III.

Animal Inoculated.—Calf 108.

Calf 108 was inoculated subcutaneously with a tissue emulsion of the original material, estimated to contain 14,500,000 bacilli, and was killed 145 days afterwards.

A few small circular patches and wedge-shaped streaks of infiltration occur close to the capsule. Faintly staining renal tubules are enclosed in their midst.

In addition to a large number of cells which have all the characters of small lymphocytes, these areas contain numerous cells which react to Pappenheim's stain for plasma cells. Some of the last-mentioned type of cells are indistinguishable from ordinary large lymphocytes; but the majority of them, which occur at the periphery of the infiltration, show the transition forms of the typical plasma cell passing into a fibroblast, and are found invading, for a short distance, the adjacent interstitial tissue. In each of the patches of infiltration referred to, several oxyphil leucocytes are found; they are not coarsely granular in eosin and methylene blue specimens, but their oxyphil reaction is so strong that some of them retain a red protoplasmic stain in Ziehl-Neelsen specimens. There is some excess of fibrous stroma in the infiltrated areas. The rest of the tissue is normal, except for the presence of an occasional, isolated, finely granular leucocyte.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 153.

Calf 153 was inoculated subcutaneously with an emulsion of the tissues of guinea-pigs which had been inoculated from Heifer 13, and was killed when very ill, thirty-nine days subsequently.

Although several specimens have been searched without the discovery of any bacilli, the lesions present in this specimen have a closer resemblance to tubercular lesions than any of those previously described.

Beneath the capsule there are minute, more or less circular areas of breaking down tissue which do not react well to protoplasmic stains. A few still smaller foci are found rather more in the interior of the gland, near to, but not involving, glomeruli. There is also a slight increase of interstitial tissue in a few places. The renal tubules which have been involved in the areas mentioned show signs of breaking down, and, in some of the larger foci, are completely disintegrated. Associated with the remains of the renal tubules are a few oxyphil leucocytes and a few plasma cells, but the areas as a whole exhibit only a slight amount of infiltration or tissue reaction. In the interstitial infiltration a few plasma cells are present.

VIRUS.—H. 18. T.T.

Animal Inoculated.—Calf 165.

Calf 165 was inoculated subcutaneously with a tissue emulsion from the original material, containing 4,500,000 bacilli, and was killed fifty-seven days subsequently.

Small wedge-shaped areas, with their base bordering upon the capsule, pass inwards for a short distance. These are packed with small lymphocytes which surround the renal tubules. Neither oxyphil leucocytes nor plasma cells are demonstrable within them. They exhibit only very slight increase of fibrous tissue. A short distance within the cortex and not connected with these areas, a scanty infiltration with plasma cells occurs at a few places in the interstitial tissue. There is no evidence of caseation in any part of the tissue.

VIRUS.—H 23. J.P.

Animal Inoculated.—Calf 365.

Calf 365 was inoculated subcutaneously with an emulsion, estimated to contain over 100,000,000 bacilli, of the original material, and was killed sixty-one days afterwards.

There is a patchy, interstitial infiltration which is not typical of tuberculosis.

No tubercle bacilli have been found.

The cells constituting the interstitial infiltration are mostly lymphocytes and fibroblasts. Very few oxyphil leucocytes are found. Amongst the fibroblasts are typical plasma cells which take a well-marked differential stain.

B. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Calf 122.

Calf 122 was inoculated subcutaneously with an emulsion estimated to contain about 1,000,000 bacilli, from the tissues of Monkey 62, and was killed sixty-four days afterwards.

Beneath the capsule are caseo-calcareous tubercles of circular outline, bordered by fibrous tissue, and containing peripherally many giant cells. Smaller tubercles, of less regular outline, are found more in the interior of the cortex; they contain many giant cells, and show little or no sign of caseation. There are also some narrow wedge-shaped patches of infiltration passing inwards from the capsule, and bearing a general resemblance to the areas of infiltration described as found in the kidneys not specifically tubercular.

In the areas last mentioned no tubercle bacilli have been found. They are present in the other lesions, but are rare. One has been found in the midst of a calcareous deposit. They occur occasionally amongst the caseous material and within giant cells.

Only a few blood capillaries containing red corpuscles are found within the infected areas.

Finely granular oxyphil leucocytes are scattered in large numbers throughout the older tubercular foci, except in the regions of complete necrosis. Of the smaller and less advanced foci some contain many leucocytes, and others very few. In the rest of the tissue also the distribution of leucocytes is irregular. A few of the renal collecting tubules are filled with them, and there are, near to these, some minute patches of infiltration, consisting mainly of leucocytes. The streaks and wedge-shaped tracts of infiltration which have been described as not specifically tubercular, do not contain many multinuclear leucocytes. Lymphocytes and plasma cells are abundant in the infiltrated areas.

Giant cells are numerous and large; the majority of them appear from the characters of their nuclei to be derived from endothelial cells. The endothelial proliferation is well marked; the fibrous tissue formation is dense, and forms a definite zone round the older tubercles.

SUMMARY.

Many tubercles, the older being caseo-calcareous. Giant cells numerous. Considerable fibrous tissue formation. Bacilli rare.

VIRUS.—B I.

Animal Inoculated.—Calf 124.

Calf 124 was inoculated subcutaneously with an emulsion, estimated to contain 9,000 bacilli, from the tissues of Pig 12, and died seventy-one days afterwards.

The largest lesions, situated in the cortex, are of irregular outline and exhibit advanced caseation with commencing calcification. In their neighbourhood is a good deal of infiltration by cells with small round nuclei and a marked increase of fibrous tissue which is spreading into the surrounding parts. The smaller lesions are of the same type, and of irregular outline, but show less advanced necrosis, whilst the earliest consists merely of an interstitial infiltration containing many cells with small round nuclei. Giant cells are numerous.

Tubercle bacilli are fairly numerous in the necrotic patches, and sometimes form a ring surrounding blood-vessels which have escaped destruction in these areas. They are also easily found within the areas of cellular infiltration and frequently occur within giant cells. The older lesions contain more tubercle bacilli than the earlier.

Deposits which appear to be old fibrin are found in the advanced lesions; they are most dense around the blood-vessels which have escaped destruction in these situations.

Some of the smaller, but centrally caseous, lesions are swarming with oxyphil leucocytes and leucocytes which

have lost their protoplasmic staining properties; within and surrounding the oldest lesions very few leucocytes are demonstrable; leucocytes are also rare in the earliest areas of infiltration. In all the localities described as "infiltrated" both small lymphocytes and plasma cells are very abundant; they are least numerous where the leucocytes are most plentiful. Many of the cells described as "small lymphocytes" are surrounded by a rim of red protoplasm in Pappenheim specimens and may be "daughter" plasma cells.

Judging by the characters of their nuclei, the giant cells appear to be of two kinds, some being derived from endothelial cells and others from renal epithelial cells; there is no evidence that any have been formed from plasma cells. There is a well marked endothelial proliferation, and in some places a tendency of these cells to run in parallel lines, giving the appearance of empty tubes cut in longitudinal section. Some of these endothelial cells are swollen and rich in protoplasm, but cells of this type occur irregularly, and do not form a definite zone in relation to the tubercular areas.

The fibrous tissue increase is prominent in relation to the tubercular areas, and also occurs to a slight extent in a few small patches in the non-tubercular parts of the interstitial tissue, and surrounding some of the blood-vessels.

SUMMARY.

Caseous, progressive tubercles, containing many bacilli.

VIRUS.—B I.

Animal Inoculated.—Calf 128.

Calf 128 was inoculated subcutaneously with an emulsion, estimated to contain 110,000 bacilli, from the tissues of Calf 102, and was killed, when ill, sixty-four days afterwards.

There are numerous tubercular foci which are caseous in their interior. The largest of these foci border upon the capsule, where their area is widest, and become gradually narrower as they pass inwards, penetrating as far as the intermediate zone which contains large arteries and veins. They are intersected by strands and islands of unaltered renal tissue. Much smaller foci are also frequent, both beneath the capsule and in the interior of the cortex; they are of irregular outline, and, with the exception of the smallest, exhibit commencing caseation; they make their first appearance in the interstitial tissue and involve the renal tubules before they involve the glomeruli.

Tubercle bacilli are rare but can be found, after careful search, in many of the lesions.

There is some extravasation of red blood corpuscles in the necrotic areas.

Oxyphil leucocytes are scattered throughout the infected areas in large numbers, and are often massed together in groups within or near sites of commencing necrosis. Small lymphocytes are quite as numerous as the oxyphil leucocytes. Plasma cells are found abundantly throughout the earlier lesions and at the periphery of the lesions when caseation is present.

Some of the breaking down renal tubules resemble giant cells; a similar appearance is caused by some of the occluded capillaries; there are other formations which may be definitely termed giant cells, but these are not numerous. At the margin of the caseating areas and in the adjacent interstitial tissue between renal tubules which remain intact, there is a proliferation of cells with oval nuclei and a considerable amount of protoplasm which does not stain by Pappenheim's method. These cells appear to be of endothelial origin. They do not form a typical epithelioid zone.

Though fibroblasts are plentiful, the amount of adult fibrous tissue is not great and does not serve to demarcate the tubercular areas.

SUMMARY.

Many caseating tubercles in which bacilli are rare.

VIRUS.—B I.

Animal Inoculated.—Calf 132.

Calf 132 was inoculated subcutaneously with an emulsion estimated to contain about 1,000,000 bacilli, from the tissues of Monkey 62, and died 35 days afterwards.

Immediately beneath the capsule and also a short distance within the cortical substance there are several small irregular areas of interstitial infiltration containing numerous cells with small round nuclei. Many of these areas are close to glomeruli, but none have been found actually involving glomeruli.

Tubercle bacilli are present in small numbers in all these areas. Some of the bacilli are intracellular. A few bacilli are found within cells of the renal epithelium. No bacilli have been found within any of the glomeruli.

Fibrin is present within a few of the blood-vessels, but has not been satisfactorily demonstrated in any of the tubercular areas, although a few patches in the latter are somewhat suspicious.

No oxyphil leucocytes are demonstrable in the lesions. The fact that the animal was found dead must be borne in mind. Nuclei are present which are not unlike those of multinuclear leucocytes, but the number of these nuclei is small. Small lymphocytes are abundant; cells resembling large lymphocytes are present, but not very numerous; unmistakable plasma cells are more numerous, especially at the margins of the areas of infiltration.

No giant cells have been found. The cells of the renal tubules are becoming detached and breaking down, but show no signs of proliferation. There is no clear evidence of a proliferation of endothelial cells.

There are young fibroblasts in the affected areas, but there is no demarcation of these areas by fibrous tissue, nor is there an increase of fibrous tissue elsewhere.

SUMMARY.

A cellular infiltration of the interstitial tissue, associated with tubercle bacilli.

VIRUS.—B. IX.

Animal Fed.—Calf 380.

Calf 380 was fed with 1 mg. of culture of B IX. and was killed 140 days afterwards.

Small caseating tubercles, containing many giant cells, are present in the cortex.

Bacilli are present, but are very rare.

SUMMARY.

Small caseous tubercles, containing very few bacilli, are present. It is noteworthy that these lesions were produced by feeding with a small dose of culture.

C. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 101.

Calf 101 was inoculated intravenously with an emulsion estimated to contain 48,000 bacilli, from the tissues of Calf 5, and was killed, when moribund, 41 days afterwards.

There are many patches of infiltration, chiefly in the cortex, which are characterised by the presence of numerous cells with round nuclei. The larger of these areas contain caseous patches and giant cells, and are partially marked off from the rest of the kidney by fibrous tissue. The smaller areas show no signs of breaking down, but send out lines of interstitial infiltration which ramify in all directions. The renal tubules are encroached upon by this infiltration much sooner than the glomeruli. In sections taken from another part of the kidney there are no lesions typical of tuberculosis, but an interstitial infiltration with small cells suggestive of that found in leukæmic kidneys.

The part of the kidney last mentioned contains no tubercle bacilli. In the other portions examined, bacilli are plentiful in the caseous areas, within giant cells, and amongst the earlier areas of infiltration.

There is old fibrin in the advanced lesions, but none elsewhere.

No oxyphil leucocytes are demonstrable in any of the specimens, but occasional nuclei are found throughout the infiltration which resemble those belonging to this type of cell. In the tubercular areas plasma cells are the chief constituents of those parts of the tubercular foci which do not show degenerative changes; they are also the chief constituent of the early interstitial infiltration found in the sections from the tubercular parts of the kidney. In addition to the large type of plasma cell, the smaller type, with a narrow rim of red staining protoplasm, is particularly abundant. Cells with small round nuclei and no demonstrable protoplasm, and which may therefore be regarded as ordinary small lymphocytes, are present, but not so numerous as plasma cells. On the other hand, the sections taken from a non-tubercular part of the kidney show that the infiltration there present consists almost entirely of small lymphocytes; only one or two very small patches of plasma cells can be found.

Cells of endothelial type play some part in the tubercular process. They are most readily recognised near the patches of commencing caseation, internal to the plasma cells. They are also found growing in some situations in parallel columns, and proliferating cells of endothelial type are occasionally seen close to, and probably originating from, the outer wall of a glomerulus. The giant cells are probably of endothelial origin.

The fibrous tissue increase is fairly general and diffuse in the tubercular areas, but does not form dense strands.

SUMMARY.

A tubercular infiltration which appears to have commenced in the interstitial tissue and not in the glomeruli. Bacilli numerous in the caseous patches and some of the less advanced areas of infiltration.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 105.

Calf 105 was inoculated subcutaneously with the same dose of the same material as Calf 101, and was killed, when moribund, 47 days afterwards.

In the cortex are found a few tubercles which are distinct from one another, but placed near together. The area of each is about that of the microscopic field with a low power (No. 4 eyepiece; 16mm. objective). The tubercles are slightly caseous in the centre, and are surrounded with and permeated by a considerable amount of adult fibrous tissue.

The tubercles contain bacilli which are found, though not in large numbers, in broken-down cells, giant cells, and the caseating foci.

Scattered throughout the tubercles and frequently forming groups are multinuclear leucocytes which take a well-marked oxyphil stain. They are all of the ordinary polymorphonuclear type, but in some the granulation of the protoplasm is rather coarser than usual. Small lymphocytes are also present. Plasma cells are the chief constituents of the marginal portions of the tubercles and infiltrate, to a slight extent, the adjacent tissue. Cells which are probably endothelial are also numerous, particularly in the inner parts of the tubercles, and the giant cells appear to have been formed from cells of this type rather than from renal epithelial cells.

There is considerable increase of fibrous tissue within and surrounding the tubercles.

SUMMARY.

Caseous tubercles more definitely circumscribed and fewer in number than those produced by the intravenous inoculation in Calf 101. Bacilli are present, but not in large numbers.

VIRUS.—H 7. C.M.
Animal Inoculated.—Calf 109.

Calf 109 received an intraperitoneal inoculation with an emulsion from the tissues of Calf 5, estimated to contain 48,000 bacilli, and died 40 days afterwards.

In the cortical substance of the kidney there are several early tubercles. These have not begun to caseate, and have only caused a small amount of destruction of the renal cells; they are characterised by an infiltration of lymphocytes and leucocytes, the former cells being more numerous than the latter. These areas are irregular in outline and are not definately bounded by fibro-blasts but are evidently spreading by way of the interstitial tissue.

Tubercle bacilli, though not numerous, are present in all the lesions.

SUMMARY.

The kidney contains numerous early tubercles.

VIRUS.—H. 7. C.M.
Animal Inoculated.—Calf 141.

Calf 141 was inoculated subcutaneously with an emulsion estimated to contain about 250,000 bacilli, from the tissues of Cow 73, and was killed 53 days afterwards.

The lesions present are of three kinds:—(1) large tubercles showing advanced caseation; (2) small tubercles which have not arrived at the stage of caseation; (3) interstitial infiltration.

Tubercle bacilli are present in all these three situations, and are very abundant in both (1) and (2). None occur elsewhere. Two bacilli are often found closely adherent to each other, but no true branching forms occur.

There is a considerable amount of fibrin, both in the older foci and in quite early lesions. In the latter situations the fibrin is of recent formation. The fibrin is always associated with numerous tubercle bacilli.

Oxyphil leucocytes of the ordinary type, but with distinct granulation, are found abundantly in all the advanced lesions, and are present, but in much smaller numbers, in the early areas of infiltration. Small lymphocytes and plasma cells are plentiful in the usual situations; many of the plasma cells are in mitotic division. The early infiltration contains a large number of plasma cells.

Endothelial cells have a tendency to break down before they have succeeded in forming a definite epithelioid layer. The renal epithelial cells also show signs of rapid disintegration in all but the earliest lesions.

In all the lesions there is new fibrous tissue formation, but the fibrous tissue does not succeed in forming a barrier to the tubercular process; both old and early lesions have a tendency to overflow into the surrounding tissue.

SUMMARY.

Rapidly progressive tuberculosis. Fibrin present. Bacilli abundant.

VIRUS.—H 8. G.C.
Animal Inoculated.—Calf 305.

Calf 305 was inoculated intravenously with an emulsion, estimated to contain nearly 730 million bacilli, from the tissues of guinea-pigs which had been inoculated from Calf 177. Calf 305 was killed, when very ill, 26 days afterwards.

Passing inwards from the capsule there is a small, somewhat wedge-shaped patch which exhibits a slight degree of infiltration. There are several other patches of infiltration in the cortex which are still smaller, and not wedge-shaped; several of these are near to blood-vessels, but none of them appear to originate from glomeruli.

In the first area of infiltration mentioned only one tubercle bacillus has been found, and that is within a renal epithelial cell. In most of the other areas one or two bacilli can generally be found, and are frequently within the renal epithelial cells. No tubercle bacilli have been found in the glomeruli. It therefore seems possible that the bacilli present have circulated through the glomerular capillaries, and subsequently escaped from the venous capillaries surrounding the renal tubes.

No fibrin is present.

Oxyphil leucocytes are very rare, but two or three are to be found in many of the lesions. In all the areas described as "infiltrated," plasma cells are present and form the most conspicuous invading element. Plasma cells are frequently associated with the outer sheath of blood-vessels, and may have travelled from that situation to the actually tubercular foci.

There is also some increase in the affected areas of fibroblasts which, as shown by their different staining properties, are not derived from plasma cells.

SUMMARY.

A slight, interstitial, cellular infiltration, associated with tubercle bacilli. No fibrin is present.

VIRUS.—H 8. S.C.
Animal Inoculated.—Calf 361.

Calf 361 was inoculated intravenously with an emulsion estimated to contain 41,500,000 bacilli, from the tissues of Calf 275, and was killed 76 days afterwards.

Small caseous tubercles are present in the cortex. They are surrounded by a zone of plasma cells and lymphocytes and contain small numbers of oxyphil leucocytes.

Tubercle bacilli are present in the lesions, but are rare.

SUMMARY.

Small, caseous tubercles, containing few bacilli, are present.

VIRUS.—H 10. B.S.

Animal Inoculated.—Calf 191.

Calf 191 was inoculated subcutaneously with an emulsion, estimated to contain $2\frac{1}{2}$ million bacilli, from the tissues of Calf 35, and was killed, when moribund, 60 days afterwards.

Large, isolated tubercles showing advanced fibroid and caseous change. Rest of tissue normal.

Tubercle bacilli are found without difficulty in the areas both of commencing and of advanced necrosis.

Finely granular oxyphil leucocytes are plentiful throughout the tubercles. Their granules are rather coarser than usual and strongly oxyphil. Occasionally a cell is found with granules so much coarser than the rest that it may be definitely called a coarsely granular oxyphil.

The distribution of small lymphocytes, plasma cells, and endothelial cells is similar to that in advanced tubercles previously described.

Summary.

Fibro-caseous tubercles, containing many bacilli.

VIRUS.—H 12. H.N.

Animal Inoculated.—Calf 319.

Calf 319 was inoculated intravenously with an emulsion estimated to contain about 100 million bacilli, from the organs of guinea-pigs which had been inoculated from Calf 189. Calf 319 was killed, when moribund, 57 days afterwards.

In the medulla there is a large area of infiltration and commencing necrosis. The cortex contains numerous smaller areas of infiltration which are not limited to any particular situations, but do not involve the glomeruli. These areas show no sign of caseation or necrotic changes, nor do they contain any giant cells.

The kidney is swarming with tubercle bacilli. In the medulla they form colonies visible with a low power of the microscope. In the cortex, though not occurring in such dense masses, large numbers are found in every microscopic field. The bacilli are all found in the interstitial tissue. None are found in either glomeruli, blood-vessels, or renal cells.

No evidence of fibrin has been found in any of the specimens.

Oxyphil leucocytes are numerous in some of the breaking down foci in the medulla, but are much less frequent in the cortex. The other cells composing the infiltration are lymphocytes, ordinary fibroblasts, and a relatively small number of plasma cells. Many of the last are in mitotic division.

Summary.

An interstitial infiltration with nothing, histologically, which can be regarded as typical of tuberculosis. It is noteworthy that tubercle bacilli are present in enormous numbers, although there is very little tissue destruction. No fibrin is present.

VIRUS.—H. 13. A.D.

Animal Inoculated.—Calf 301.

Calf 301 was inoculated subcutaneously with an emulsion, estimated to contain approximately 540,000,000 bacilli, from the tissues of guinea-pigs which had been inoculated from Calf 129. Calf 301 was killed, when very ill, thirty-three days afterwards.

In the cortex there are many small cellular foci in which the renal tubules are either entirely obliterated or only indicated by gaps half filled with broken down epithelial cells. There are also numerous patches which are still smaller and more irregularly outlined, and appear to represent earlier stages of the foci above mentioned. In these earlier foci most of the renal tubules are preserved, and the predominant feature is the presence of new elements in the interstitial tissue, but there is almost always some indication of damage to the renal parenchyma. The sections examined have been taken from two portions of the kidneys, and in no instance have the earliest lesions been found to involve the interior of a glomerulus. In the relatively larger lesions the glomeruli survive unaltered longer than the tubules, and in only one of them is the disintegrated appearance of a glomerulus such as to render it doubtful whether the infection has spread from the glomerulus to the surrounding tissue rather than from the surrounding tissue to the glomerulus.

Tubercle bacilli are found in all the lesions, and are numerous in all except the earliest. Much the greater number of them are found within cells or fragments of cells which can be recognised as belonging to the renal epithelium. In many of the smaller lesions this is the only situation in which bacilli occur. Within the doubtful glomerulus referred to above there are several bacilli. Otherwise, the glomeruli are remarkably free from bacilli. Many glomeruli found in the middle of the more advanced areas contain none, whereas in the adjacent cells belonging to renal tubes bacilli are numerous. No bacilli have been found within any of the glomeruli in the non-tubercular parts of the gland. A further point with regard to the intracellular bacilli is that, whereas in the earlier lesions they occur singly, in the later lesions they are found in small groups ; as many as twenty are often present within a single cell or cell fusion. There is therefore strong evidence that intracellular residence is the condition, in these specimens, which has proved most favourable to the multiplication of the bacilli.

No fibrin has been found.

Oxyphil leucocytes are present in most of the infected areas, but only in very small numbers. Small lymphocytes are present in rather larger numbers. Plasma cells are conspicuous and abundant in all the lesions, and in the earlier ones constitute almost the whole of the interstitial infiltration. Many of them are in mitotic division. There are also small streaks of infiltration with plasma cells in places which are not recognisably tubercular. Many of the plasma cells are becoming narrowed and elongated into fibroblasts.

There is no evidence of any considerable proliferation of endothelial cells. In the older lesions it is sometimes impossible to decide whether broken down cells and cell fusions are of endothelial or epithelial origin ; there is, however, no typical transition of endothelial cells into an "epithelioid" type. Some of the cell fusions may perhaps be called giant cells, but there are no typical giant cells such as are found in chronic tubercular lesions.

There is no attempt at a fibrous tissue demarcation of the tubercular foci.

Summary.

Disseminated, progressive tubercles. Bacilli numerous; no fibrin.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 325.

Calf 325 was inoculated subcutaneously with an emulsion from the tissue of Calf 301, containing, approximately, 500,000,000 bacilli. Calf 325 was killed, when moribund, 24 days afterwards.

The kidney contains many small tubercles which appear to have originated in the interstitial tissue. They are infiltrated with leucocytes and lymphocytes, and are commencing to break down. There is no evidence of any demarcation of these areas from the surrounding tissue.

Tubercle bacilli, generally straight and rather short, are numerous in all the lesions.

Summary.

Progressive miliary tuberculosis. Bacilli numerous.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 121.

Calf 121 was inoculated subcutaneously with an emulsion, estimated to contain over 360,000,000 bacilli, of the original material, and was killed, when ill, thirty-six days afterwards.

In the sections examined not more than half a dozen tubercle bacilli have been found. These all occur in the same situation, a small patch of interstitial infiltration in the intermediate zone between cortex and medulla. In the same area oxyphil leucocytes are present, but not abundant; small leucocytes are much more numerous, and there are also some plasma cells.

Summary.

Slight tuberculosis. This case illustrates the high resisting power of the bovine kidney. The dose inoculated into this animal was very large, and the bacilli were known to be highly virulent.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 125.

Calf 125 was inoculated subcutaneously with the same dose of the same material as Calf 121, and was killed, when very ill, twenty-three days afterwards.

Throughout the tissue there is a general and advanced interstitial infiltration consisting of small lymphocytes, fibroblasts, and a variable number of plasma cells. In a few places the renal tubes are entirely obliterated, and there is commencing necrosis.

In the last-mentioned areas tubercle bacilli are present. None have been found elsewhere.

Oxyphil leucocytes are found in groups in or near the areas of commencing necrosis, and in most of these situations bacilli are also found. Plasma cells are more numerous at the periphery of the tubercular foci than in the other areas of infiltration which are not specifically tubercular.

Summary.

A good deal of interstitial infiltration, but only a slight amount of necrotic change. Tubercle bacilli only found in or near the areas which show necrotic change.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 197.

Heifer 197 was inoculated subcutaneously with an emulsion, estimated to contain about 85,500,000 bacilli, from the tissues of Calf 121, and was killed, when very ill, fifty-one days afterwards.

Large circumscribed tubercles, circular in outline and showing advanced caseation with slight calcification; also wedge-shaped areas of infiltration.

The former contain tubercle bacilli in scanty numbers; the latter do not contain any.

In both types of lesion there is an extensive leucocytic infiltration. In the tubercular foci the renal tubules which have escaped destruction at the margin of the lesions are filled with leucocytes. In the infiltrated areas there are also several renal tubules which are filled with leucocytes, whilst many others contain a coagulated, hyaline deposit. Plasma cells and small lymphocytes occur in both types of lesion, but plasma cells are much more numerous in the tubercles, where they form a definite boundary zone of cells passing into fully formed fibroblasts.

Summary.

Caseous, slightly calcareous, circumscribed tubercles. Bacilli scanty. Leucocytes abundant.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 243.

Heifer 243 was inoculated subcutaneously with an emulsion, estimated to contain about 4,500,000 bacilli, from the tissues of Heifer 197 and was killed when very ill, sixty-one days afterwards.

Isolated tubercles similar to those in Heifer 197, but with more advanced calcification.

Tubercle bacilli are present in these tubercles, but are rare.

Leucocytes only occur in small numbers within the tubercles and are not present in other parts of the tissue. Plasma cells and lymphocytes as in tubercles of Heifer 197. In these kidneys no large patches of infiltration have been found such as those described in Heifer 197 : but there are occasionally found, amongst parts of the tissue otherwise normal, minute groups of plasma cells.

In some of the tubercles there is a fairly well defined epithelioid zone internal to the plasma cells.

SUMMARY.

Isolated, caseo-calcareous tubercles, containing few bacilli.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 355.

Calf 355 was inoculated subcutaneously with an emulsion of the tissues of Calf 273, containing, approximately 3,000,000 bacilli. Calf 355 was killed when very ill, 43 days afterwards.

There are many streaks of infiltration which contain numerous plasma cells. A few definite tubercles are also found. These are infiltrated with lymphocytes, leucocytes, and plasma cells, and sometimes contain a few giant cells ; they are not caseous.

No tubercle bacilli have been found.

SUMMARY.

Slight tuberculosis.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 529.

Calf 529 was inoculated intravenously with an emulsion from the tissues of Calf 539, containing, approximately, 1,000,000,000 bacilli. Calf 529 died 13 days afterwards.

There is a slight degree of interstitial infiltration, but no histologically tubercular lesions have been found in the specimen examined.

Tubercle bacilli are found in scanty numbers in the interstitial tissue, and a few are present within glomeruli.

SUMMARY.

Bacilli are present in scanty numbers but there are no definite tubercles.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 555.

Calf 555 was inoculated subcutaneously with an emulsion from the tissues of Calf 553, containing, approximately, 150,000,000 bacilli. Calf 555, was killed, when moribund, 58 days afterwards.

Large, caseous tubercles are present. They are of irregular, ramifying outline, and contain a fibrinous deposit.

Tubercle bacilli, most of them straight and rather short, are very numerous in the lesions.

SUMMARY.

Acute, progressive, tuberculosis ; bacilli numerous.

VIRUS.—H 19. S.W.

Animal Inoculated.—Calf 159.

Calf 159 was inoculated subcutaneously with an emulsion estimated to contain 1,500,000 bacilli, of the original material, and was killed 50 days afterwards.

These kidneys contained pale wedge-shaped areas in the cortex. One of these has been microscoped. It contains no tubercle bacilli and exhibits an interstitial infiltration which is not typical of tuberculosis. The cells composing this infiltration are small lymphocytes and connective tissue corpuscles. As similar lesions have been described in the first part of this report, it is not necessary to describe this lesion in further detail.

Other lesions found in these kidneys are definitely tubercular.

These lesions consist of large areas in which the normal renal tissue is almost entirely obliterated by the presence of other cellular elements. The areas show commencing

necrosis, contain several giant cells, and are more or less completely bounded by fibrous tissue.

Tubercle bacilli are found in small numbers in the necrosing areas, within giant cells, and, occasionally, within a cell belonging to a renal tubule which has been included within the margin of a tubercular focus.

Oxyphil leucocytes are occasionally found within these areas, but are very rare. Both small lymphocytes and plasma cells are densely aggregated at the periphery of the foci. More in the interior of the foci, cells of endothelial type are abundant.

It is possible that these foci were originally patches of infiltration not associated with tubercle bacilli, and that they subsequently proved the most favourable site for tubercular infection.

SUMMARY.

Early tubercles containing giant cells and partially bounded by fibrous tissue. Bacilli not numerous.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 399.

Calf 399 was inoculated intravenously with an emulsion of the lesions of Guinea-pig 200, containing over 700,000,000 bacilli, which was one of a series inoculated from Calf 291. Calf 399 was killed when very ill, 34 days afterwards.

In the interstitial tissue there are patches of infiltration, some of which have involved and partially obliterated groups of renal cells. The cells in the infiltrated tissue are mainly plasma cells and lymphocytes. No fibrin is present. The glomeruli are normal.

Bacilli are present in the infiltrated interstitial tissue, in the glomeruli, and in the blood-vessels.

SUMMARY.

Tubercle bacilli are present throughout the tissue but have produced only slight tissue damage.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 749.

Calf 749 was inoculated intravenously with 46 mg. of culture isolated from Calf 293, and was killed when very ill, 24 days afterwards.

Very little histological change is noticeable, but there are a few slight patches of infiltration in the interstitial tissue. Tubercle bacilli are only found in very small numbers.

SUMMARY.

A few bacilli are present but have caused very little tissue reaction.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 551.

Calf 551 (a bull-calf 10 months old) was inoculated intravenously with 100 mg. of culture isolated from Calf 417, and died 21 days afterwards.

The only abnormality present is an occasional small collection of lymphocytes and leucocytes in the interstitial tissue.

Tubercle bacilli are rare but are occasionally present in these situations.

SUMMARY.

A few bacilli are present but have produced no evidence of tissue damage.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 831.

Calf 831 was inoculated intravenously with an emulsion of the tissue of Calf 551 containing about 1,000,000,000 bacilli, and died 17 days afterwards.

There are many patches of interstitial infiltration, and in a few situations there are definite tubercles which are surrounded by a zone of lymphocytes and leucocytes and contain in their centre partially obliterated renal cells. In all these lesions tubercle bacilli are numerous. They are also occasionally found within glomeruli and in blood-vessels.

SUMMARY.

Bacilli are numerous and have led to the formation of many lesions which involve the renal epithelium to a slight extent.

VIRUS.—H 25. A.T.

Animal Inoculated.—Calf 859.

Calf 859 was inoculated intravenously with an emulsion of the tissue of Calf 831 containing about 2,000,000,000 bacilli, and died 19 days afterwards.

There is a small amount of cellular infiltration in some parts of the interstitial tissue, but no definite tubercles have been found. The glomeruli appear to be normal.

Tubercle bacilli are present, but only in small numbers. They occur in the interstitial tissue, within glomeruli, and within blood-vessels.

SUMMARY.

Tubercle bacilli are present, but have produced very little cellular reaction.

V. EXPERIMENTAL TUBERCULOSIS OF LYMPHATIC GLANDS.

A. PRELIMINARY OBSERVATIONS ON GLANDS NOT TUBERCULAR.

Cow 217.

Cow 217 was a normal milch cow, not used for experimental purposes, and was killed 106 days after the last tuberculin test, which proved negative. The liver contained a large number of flukes.

The following is a detailed description of a bronchial gland which was selected as representative of normal tissue.

Lymphocytes.—Pappenheim has pointed out that his stain for plasma cells also acts as a differential stain for lymphocytes. This stain is particularly useful for picking out the large lymphocytes which contain a large amount of basophil, non-granular, protoplasm. These cells stain very much like plasma cells, the protoplasm being bright red, and the chromatin elements of the nuclei deep purple. The distribution of these cells in this gland is as follows. In the inner portion of the capsule they occur irregularly and often form small groups, but their total number is only a small proportion of the cells present; some of them are undergoing nuclear mitosis. In the fibrous trabeculæ which pass inwards from the capsule, they only occur in very small numbers. In the denser masses of cortical lymphoid tissue, their distribution is irregular; there are some situations which contain groups of considerable size, and there are large areas which contain none. Passing inwards towards the medullary substance, the small islands of lymphoid tissue are found to be almost entirely composed of these cells. In the lymph sinuses they occur occasionally, not in groups, and are lying apparently free. Throughout the gland a few of these large lymphocytes are to be found which exhibit signs of degeneration. Their nuclei are then much larger, poorer in chromatin, and often contain a large red globule. With the exception of this globule and the darkly-stained remnants of chromatin, the colour of the nucleus is pale green; their protoplasm is diminished in amount and broken up, but what remains still takes a definitely red stain. The small lymphocytes which are present throughout the glands are characterised in Pappenheim specimens by the deep and uniform stain of their nuclei. Round the smallest, a narrow rim of red protoplasm is sometimes, but not always, demonstrable. Intermediate forms between the small and large lymphocyte are everywhere to be found; these cells possess the spherical, deeply and uniformly staining nucleus of the small type, but have a much larger amount of protoplasm.

Oxyphil Cells.—(a) Coarsely granular. It is known that rather small coarsely granular eosinophil cells occur normally in most lymphatic glands, and that their numbers and distribution are highly irregular. In this gland they occur in exceptionally large numbers. Perhaps this fact is to be associated with the presence of parasitic infection. In most parts of the capsule they form dense masses; in many places, as many as five out of every six cells present are of this type. In the fibrous trabeculæ passing inwards from the capsule they are present in enormous numbers, and amongst the trabeculæ more in the interior of the gland they occur in large but variable numbers. Amongst the cortical part of the lymphoid tissue from about 6 to 20 are to be found in most microscopic fields (No. 8 eyepiece; 4 mm. objective), the numbers being greater in proximity to the trabeculæ. Amongst the smaller islands of lymphoid tissue more in the interior of the gland they occur with much less frequency. They are present in the lymph sinuses. In specimens stained by Pappenheim's method, the protoplasm of these cells remains unstained and no granules are visible; their nuclei take a lighter and more greenish stain than the nuclei of lymphocytes. The above description of the relative distribution of the coarse eosinophils and the lymphocytes is based on the comparison of a Pappenheim specimen and an eosin and methylene blue specimen prepared from the same piece of tissue. (b) Finely or obscurely granular cells. Ordinary polymorphonuclear and mononuclear oxyphil cells also occur, but are very rare. They are generally found within or near the lymph sinuses.

Vascularity.—Very occasionally a red blood corpuscle is seen lying free in or near the lymph sinuses. The contents of the blood vessels are normal.

Endothelial Cells.—Endothelial cells are generally easy of recognition when their flat surface lies parallel to the plane of section. In Pappenheim specimens their nuclei are pale green, and their protoplasm is either colourless or only exhibits a faint red tinge. Sometimes, however, it is not possible to distinguish between an endothelial cell and a large degenerate lymphocyte the nucleus of which has become swollen and which has lost most of its protoplasm. Features which help in the differentiation are the tendency of the endothelial cell to have an irregular branching outline and its tendency to form groups in which the individual cells are in actual contact with one another.

Right Prescapular Gland.—The lymphocytes are similar in character and distribution to those in the bronchial gland. Coarsely granular oxyphil cells are again very abundant, especially in the interstitial tissue, but do not occur in such enormous numbers as in the previous gland. The nuclei of these cells are, as a rule, more regular in outline than those of the ordinary finely granular polymorphonuclear leucocyte; but horse-shoe, trilobed, and intermediate forms are found. An occasional finely granular oxyphil leucocyte is found in the lymph sinuses. The characters of the vessels and endothelial cells are similar to those of the bronchial gland.

Mesenteric Gland.—Like the bronchial gland, this gland contains enormous numbers of rather small coarsely granular eosinophil cells. Their numbers are greatest in the part of the gland nearest to the hilum; in this part of the tissue they occupy the entire field, and, with the exception of the constituents of the blood vessels and fibrous trabeculæ, very few cells of any other type are present. As we pass from this region to the cortex, there is a rapid diminution in their numbers. Finely granular oxyphil cells occur in larger numbers than in the two glands previously described; they occur singly, and never form definite aggregations; their total number is not very great. Several large cells are found filled with granules which take a purple stain in eosin and methylene blue specimens. In other respects the gland is similar to the two described above.

Heifer 96.

Not used for experiment, but gave two suspicious tuberculin reactions, the last on October 29th, 1903, the inoculation being made on right side of neck. Rise of temperature, 1° C. Killed seven days afterwards. No tubercular lesions found.

Bronchial Gland.—The lymphocytes and endothelial cells present no differences from those of Cow 217. The coarsely granular eosinophil cells are fairly numerous, and are distributed throughout the gland, but are much less abundant than in Cow 217. Some of the largest groups of them are found amongst the fibrous trabeculæ. Finely granular leucocytes occur, but are rare. In several parts of the gland there are minute hæmorrhages.

Left Prescapular Gland.—There is nothing abnormal about the lymphocytes or endothelial cells. Coarsely granular eosinophil cells are scattered throughout the gland, in slightly smaller numbers than in the bronchial gland. There is a definite infiltration of ordinary finely granular multinuclear leucocytes; this infiltration has not extended to the denser cortical nodules of lymphoid tissue, but is conspicuous in the interior part of the gland,

where the leucocytes form in many places large irregular aggregations. There is no diminution of the number of coarsely granular eosinophils in these infiltrated areas. Associated with the leucocytic infiltration is an extravasation of red blood corpuscles.

Mesenteric Gland.—Coarsely granular eosinophils are fairly numerous, no finely granular oxyphils have been found. There are no vascular or other abnormalties.

Calf 135.

Killed, October 29th, 1903, 159 days after subcutaneous inoculation with emulsion from mesenteric glands of the virus H 12. H.N. Tuberculin was inoculated 19 hours before death on the right side of the neck. Rise of temperature, 2·4° C. The following glands were examined as examples of non-tubercular glands in an infected animal which had just previously reacted to tuberculin.

Right Prescapular Gland.

Lymphocytes.—In sections stained by Pappenheim's method the number of lymphocytes which take a red protoplasmic stain is remarkably small. I have investigated this point carefully and have satisfied myself that it is not due to some accidental defect in the staining technique; I think it probably indicates that the cells which fail to stain have undergone some chemical modification of their protoplasm. The lymphocytes which take up the red protoplasmic stain appear normal; there are not many which exhibit degenerate nuclei; a few are in mitotic division, but not more than are usually found in lymphatic glands. The red staining lymphocytes occur both in the cortical and the medullary portions of the gland; but, whereas in the normal glands above described these cells occur in relatively much greater numbers in the medullary than in the cortical lymphoid nodules, in this specimen their numbers in the medullary portion are, both relatively and absolutely, particularly small.

Oxyphil Cells.—Coarsely granular eosinophils occur in small numbers throughout the gland. There is a very marked increase in the finely granular oxyphils, particularly in the lymph sinuses, many of which are packed full of them. They are not, however, very uniformly distributed. From the lymph sinuses they are seen passing into the lymphoid tissue.

Vascularity.—The most prominent feature about this gland is that it is engorged with red blood corpuscles. They occur all over the gland, filling many of the lymph sinuses and overflowing into the lymphoid nodules. The engorgment is greatest in the medullary portion. Minute deposits of early fibrin are demonstrable in a few situations. The blood vessels are dilated; the contents of the larger vessels, as seen in section, do not exhibit any signs of leucocytosis.

Endothelial Cells.—No alteration observable.

Left Popliteal Gland.

The number of lymphocytes with protoplasm which stains red by Pappenheim's method is decidedly below the normal, but is greater than the number found in the right prescapular gland. Coarsely granular oxyphils are present in small numbers. Finely granular oxyphils are less abundant than in the right prescapular gland, but are numerous in patches, both in the lymph sinuses and in the midst of the lymphoid tissue. There is no hæmorrhagic exudation.

Mesenteric Gland.

The number of coarsely granular oxyphils is rather greater than in the left popliteal gland, and the number of finely granular is rather less. In other respects the characters of this gland are similar to those of the left popliteal gland.

B. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Cow 18.

Cow 18 received an intramammary inoculation of bacilli, contained in an emulsion of the organs of guinea-pigs which had been inoculated with original material, and was killed 35 days afterwards.

Supramammary Gland.—With the exception of a few islands and strands of lymphoid and of fibrous tissue, the gland substance has been replaced by necrotic material which exhibits small points of calcification.

Tubercle bacilli are present in enormous numbers and are universally distributed. In some places they form groups visible with a low power.

In the areas which have escaped destruction there are a few cells which resemble ordinary large lymphocytes; in association with these and occurring in greater numbers are cells with all the characters of plasma cells. In the parts which are not too broken up for cells to be differentiated, multinuclear leucocytes occur, but are distinctly rare; very few are found within blood vessels. The gland is well supplied with blood vessels, which are conspicuous even in necrotic regions. In Kochel specimens a good deal of the deposit demonstrated appears to be fibrinous, but the tissue is too necrotic to make a good preparation.

SUMMARY.

Almost complete obliteration of the gland tissue. Bacilli present throughout in enormous numbers.

VIRUS.—B I.

Animal Inoculated.—Cow 40.

Cow 40 received an intramammary inoculation with an emulsion of a primary culture raised from G.P. 14 which had been inoculated with original material. The culture was 57 days old. Cow 40 was killed, "in fair condition," 92 days afterwards.

The Right and Left Supramammary Glands have both been examined, and are found to be identical in microscopic characters. They required decalcification. They are densely fibrous and calcareous in many parts, somewhat œdematous in others. Caseous patches occur here and there, and giant cells are very numerous and as large as 85μ. In the arteries there is some endarteritis and marked periarteritis, with hyaline degeneration of the outer coat. In both glands bacilli are fairly numerous.

The Right Iliac Gland is œdematous, containing a few giant cells and some patches of slight caseation; there is no calcareous degeneration. A few bacilli have been found in it.

The Left Iliac Gland required decalcification, and is

found microscopically to contain dense ramifying strands of tissue, which have undergone fibroid and calcareous transformation. There are also numerous caseous foci. The giant cells are numerous and large, measuring from 50 to 80μ; tubercles are to be found in various stages of development, some of them containing a central group of large protoplasmic cells which have not undergone fusion or degeneration, some a central group of several giant cells, and others exhibiting advanced caseation. There is a general increase of fibrous tissue, and also thickening of the arterial walls. Some portions of the gland, more especially towards the hilum, are highly œdematous; these areas presented in the post-mortem room a brownish gelatinous appearance. Bacilli occur in considerable numbers in the caseous foci; they have also been found in small numbers in close proximity to the more fibrous and calcareous parts, not infrequently in giant cells.

SUMMARY.

Caseation; giant cells numerous; very dense and extensive calcareous deposits. Bacilli fairly numerous.

VIRUS.—B I.

Animal Inoculated.—Cow 74.

Cow 74 received an intramammary inoculation with an emulsion of the udder of Cow 40, which showed advanced calcareous lesions and contained very few bacilli. Three tuberculin tests made on Cow 74 after inoculation gave no febrile reaction, but well-marked local swelling; the animal died 299 days after inoculation.

Supramammary Gland.—About one-third of the total area of the section appears normal. The lesions are varied in character and irregular in distribution. These are (1) circumscribed tubercles caseous nearly throughout and with very early calcareous deposits, (2) irregular patches of necrotic tissue, (3) smaller areas where the cells stain badly, but which are not definitely necrotic.

Tubercle bacilli are present in enormous numbers. Compared with the tissues examined from other bovines, a striking feature of this gland is that the amount of tissue destruction is small relatively to the masses of bacilli present. In most of the necrotic areas the bacilli form colonies visible with a low power, but in the circumscribed caseo-calcareous tubercles the number of bacilli is less. In areas of commencing tissue disintegration the bacilli are also so abundant as to frequently form colonies visible with a low power. Bacilli are also abundant amongst and within cells which retain their protoplasmic outline and nuclear staining properties. The bacilli exhibit an exceptional capacity for multiplying within cells; as many as a dozen bacilli are often found within a single cell, and when a cell has lost its protoplasmic boundaries, a zone of bacilli is often found completely encircling the nucleus. The cells which most frequently contain bacilli are cells of endothelial type, and degenerate lymphocytes. Bacilli have not been found within blood vessels.

The protoplasm of the lymphocytes reacts to Pappenheim's stain, but does not take as deep a colour as in normal glands. Eosin and methylene blue preparations are not satisfactory, but are sufficiently good to show that oxyphil leucocytes are present in small numbers only; the number within blood vessels is not noticeably above the normal. The tissue is richly supplied with blood vessels. Fibroblasts are numerous external to the necrotic foci; some of them appear to have been derived from plasma cells. There is also a formation of fibrous tissue of the adult type, but it is not dense; it occurs partly in association with the periphery of the tubercles, and partly as a diffuse infiltration. Fibrin is present both in the necrotic areas and in some of the places not marked by tissue destruction, where there are enormous numbers of bacilli. Most of it is not very recent, but there is some recent fibrin in the blood vessels.

Mesenteric Gland.—More advanced than the sections of the supramammary gland. Most of the normal tissue is replaced by irregular caseo-calcareous tracts between which there is an abundance of fibrous tissue.

In numbers and distribution of bacilli, and in histological details, this gland resembles the supramammary gland. There is less evidence of recent fibrin.

SUMMARY.

Progressive tuberculosis. The amount of tissue destruction is notably small, relatively to the enormous number of bacilli present.

VIRUS.—B I.

Animal Inoculated.—Calf 132.

Calf 132 was inoculated subcutaneously with about 1,000,000 bacilli from the tissues of Monkey 62, and died 35 days afterwards.

Mediastinal Gland.—Necrotic patches, small and isolated in one portion of the gland, in another portion extensive and confluent. No giant cells.

Tubercle bacilli are found in large numbers in all the necrotic foci, and are also generally distributed in the parts of the gland where tissue destruction has not commenced. In the areas of earliest invasion a large number of the bacilli are within cells, particularly endothelial cells and breaking-down lymphocytes. I have not noticed any within a lymphocyte the protoplasm of which gives a good basophil reaction.

Pappenheim specimens stain faintly and with difficulty. This is often the case with tissues of animals found dead, and also in acutely infected tissues. Large protoplasmic lymphocytes are still found abundantly in the areas not necrotic; the number of them which are in mitotic division is distinctly larger. Small lymphocytes are also very abundant, and survive for a longer period in the necrosing areas than the larger lymphocytes. A few coarsely granular eosinophils are demonstrable in the less extensively affected parts. Finely granular oxyphils are present throughout the tissue in numbers which, though not very great, are decidedly abnormal; they are also present within intact blood-vessels in numbers well above the normal. There are no definite aggregations of them, or relative increase in their numbers, in association with the tubercular foci. Blood-vessels are numerous and dilated; there is no diminution in their number in the caseating areas. There is no evidence of transition from ordinary endothelial cells to cells of "epithelioid" type. The tubercular foci are not characterised by the epithelioid type of cell, nor do they contain giant cells. In the regions of extensive tissue alteration which are in the stage prior to actual necrosis fibroblasts are abundant; they are growing irregularly in every direction, with no formation into definite bundles and no tendency to demarcate the infected from the non-infected areas. Amongst them are cells which, in Pappenheim specimens, have red protoplasm and a purple nucleus and the compressed rectangular outline which is assumed by plasma cells when in process of transition into fibroblasts. Fibrin is abundant but not very recent.

SUMMARY.

Acute, progressive infection. No giant cells. Fibrin present. Bacilli abundant.

VIRUS.—B I.

Animal Fed.—Calf 146.

Calf 146 was fed with 5,000,000 bacilli contained in the milk of Cow 64, and was killed 36 days afterwards.

Mesenteric Glands.—Three specimens have been examined. They are all caseous and in one of them the tissue is almost completely obliterated. In the less extensive lesions the caseous areas ramify in all directions and show no tendency to be limited by a fibrous barrier.

Tubercle bacilli are numerous and are evidently multiplying in the older lesions.

SUMMARY.

Advanced, progressive caseation. Bacilli numerous.

VIRUS.—B I.

Animal Fed.—Calf 154.

Calf 154 was fed with 10,000,000 bacilli contained in the milk from Cow 64, and was killed 36 days afterwards.

Mesenteric Glands.—Two specimens have been examined. One of these is normal; the other contains extensive caseous areas which ramify throughout the gland substance. At the margin of these areas there are many fibroblasts, but there is no definite layer of fibrous tissue. The tubercles, even where caseous, often contain capillaries filled with red corpuscles.

Tubercle bacilli are present in the tubercles. but are not very numerous.

SUMMARY.

Progressive, caseating lesions, less rich in bacilli than those found in Calf 146. The lesions are vascular.

VIRUS.—B I.

Animal Fed.—Calf 156.

Calf 156 was fed with 5,000,000 bacilli contained in the milk of Cow 64, and was killed 36 days afterwards.

Mesenteric Glands.—The two specimens examined both contain extensive, ramifying, caseous tracts. The structure of the lesions is similar to that found in the gland of Calf 154. The vascularity of the tubercles, even in caseous areas, is again noticeable.

Tubercle bacilli are found in fairly large numbers in the lesions.

SUMMARY.

Progressive, caseating tuberculosis. Bacilli fairly numerous. The vascularity of the lesions is noteworthy.

VIRUS B II.

Animal Inoculated.—Cow 4.

Cow 4 received an intramammary inoculation with an emulsion of the original material, and was killed 34 days afterwards.

Supramammary Gland.—Similar to the supramammary gland of Cow 18.

As regards distribution of bacilli, scarcity of demonstrable oxyphil leucocytes, and marked vascularity, this gland is similar to that of Cow 18. There is a copious fibrinous exudate and more evidence of very recent fibrin formation than in the gland of Cow 18. In many places the fibrin filaments form an extremely delicate network.

SUMMARY.

Similar to the corresponding glands of Cow 18.

VIRUS.—B II.

Animal Fed.—Calf 50.

Calf 50 was allowed to suck Cow 500 for 31 days and afterwards sucked Cow 18 for 10 days; the udders of Cow 500 (B 11.) and Cow 18 (B 1.) had been infected by intramammary inoculation. Calf 50 was killed 151 days after the commencement of experiment.

Mesenteric Glands.—The glands were greatly enlarged and densely calcareous, necessitating decalcification before microscopic examination could be made. They exhibit extensive areas of caseo-calcareous material. Some of these areas are circumscribed, but the majority of them are confluent. At their periphery there is a marked increase of fibrous tissue. Giant cells are numerous and large, measuring from 52 to 64μ in their longest diameter. Small blood-vessels, containing red corpuscles, are noticeable even in the caseous areas.

In most of the giant cells bacilli, varying in number from one to three or four, are seen. They may be situated in any portion of the cell, amongst the nuclei, in the centre, or at the non-nuclear periphery. Occasionally a larger group of bacilli, staining imperfectly, is found in the centre of a giant cell. Bacilli also occur, but only in scanty numbers, in other parts of the lesions. The bacilli are generally slightly curved and vary in length from 2·5 to 3·5μ.

SUMMARY.

Caseo-calcareous lesions of the chronic type.

VIRUS.—B II.
Animal Inoculated.—Heifer 66.

Heifer 66 was inoculated subcutaneously with an emulsion estimated to contain rather over 1,000 bacilli, from the tissues of Calf 22, and was killed 148 days afterwards. The tuberculin test, made twenty-four days before death, gave a rise of 2·5° C.

Left Prescapular Gland.—Contains many caseo-calcareous tubercles circumscribed by fibrous tissue. Giant cells numerous and large.

Tubercle bacilli are to be found in some of these areas, but are very rare.

Large lymphocytes are present in normal numbers in the non-tubercular parts of the gland, and stain differentially. There is no evidence that they take part in giant cell formation; their nuclei are generally smaller and more rounded than those of giant cells, and their protoplasm is more definitely red. Occasionally, however, two or three lymphocytes are found, the protoplasm of which has partially fused. Within the circumscribed tubercles, cells which resemble lymphocytes in morphology and staining properties are not altogether absent, but are rare; amongst the fibrous boundaries of these tubercles, cells of this type are much more common. Granular oxyphil leucocytes are very definite, and the granules are of fairly large size. But in this specimen the granules of the ordinary multinuclear leucocyte are unusually prominent, and it is probable that not more than a few, if any, of the granular cells represent the typical coarse eosinophil cell found in normal glands. Multinuclear leucocytes are most numerous in the centres of the tubercles and in the interstices of the fibrous tissue surrounding the tubercles. They are present in the neighbourhood of many of the giant cells, and I have found one within a giant cell. Though present in the non-tubercular parts of the section, they are much more numerous in the affected areas. The vascular supply is irregular, and, on the whole, not very abundant; there is no extravasation of red corpuscles within the fibrous boundaries of some of the tubercles. Cells of epithelioid type are conspicuous in the tubercles between the fibrous and the necrotic zones, and in association with the giant cells. In van Gieson specimens there is found a considerable increase of adult fibrous tissue, particularly in the boundary zone of the tubercles. In Pappenheim specimens this zone contains large numbers of cells which have all the characters of plasma cells passing into fibroblasts. There is commencing calcification in the centre of the larger tubercles.

Summary.

Caseo-calcareous tubercles, surrounded by fibrous tissue. Bacilli very rare.

VIRUS.—B II.
Animal Inoculated.—Cow 500.

Cow 500 received an intramammary inoculation with an emulsion of original material, and was killed, when very ill, thirty-two days afterwards.

External Iliac Gland.—Several small isolated patches of cells commencing to break down.

Bacilli are found in these patches in moderate numbers and also occur in small groups amongst cells which show little or no necrotic change. The bacilli are not scattered throughout the gland but are confined to these particular sites.

The specimen (an old one hardened in Müller alone) is not suitable for the differential staining of lymphocytes. A few coarse eosinophils can be seen. Finely granular oxyphils are rather plentiful in some of the lymph sinuses and are present in pathologically large numbers in the intact blood vessels. They are not noticeably associated with the tubercular foci. The gland is rich in blood-vessels, but there is no hæmorrhagic exudation. The special interest of the specimen is that it shows extremely well the effect of the early invasion of virulent tubercle bacilli. Practically every place where bacilli occur is surrounded by fresh fibrin; the fibrin occurs in no other situations.

Summary.

Early lesions of an acute type, showing fibrin associated with recent deposits of bacilli.

VIRUS.—B III.
Animal Fed.—Calf 116.

Calf 116 was killed 92 days after commencement of feeding experiment (sucking Cow 68). Tuberculin test, seven days before death. Rise, 1·6° C.

Mesenteric Gland.—Large numbers of giant cells occurring singly and in groups, in various parts of the gland. A few fairly large calcareous deposits surrounded by a zone of tissue of epithelioid type, without any intermediate caseous area.

Bacilli are by no means infrequent. A few are found in a great many of the giant cells, and in the areas near to giant cells, where there is a tendency to cell fusions. Several are demonstrable embedded in the calcareous deposits. The presence of so many bacilli is noteworthy; in tissues of this histological type bacilli are generally very rare.

Large lymphocytes are numerous and stain well. Many of them are in mitotic division. They occur quite close to the calcareous deposits and also in the neighbourhood of giant cells, but without appearing to take part in their formation. Oxyphil leucocytes are somewhat frequent, both in the tubercular areas and in the rest of the glands. The periphery of the tubercular areas does not contain adult fibrous tissue, but exhibits an irregular proliferation of spindle-shaped cells in which both endothelial cells and plasma cells appear to take part.

Summary.

Tubercles of a chronic type, histologically; but containing relatively large numbers of bacilli.

VIRUS.—B IV.

Animal Inoculated.—Calf 140.

Calf 140 (bull—10½ weeks old) received the following subcutaneous inoculation. (1) 100,000 bacilli contained in an emulsion of the tissue of Calf 120; (2) 160 days afterwards, 25 mg. of culture of B IV.; (3) 77 days afterwards, 50 mg. of culture of B IV.; (4) 132 days afterwards, 1 mg. of culture of B IV.; (5) 114 days afterwards, 100 mg. of culture of B IV; (6) 85 days afterwards, 229 mg. of culture of B IV. The animal was killed in good health 41 days after the last and 609 days after the first inoculation.

Right Prescapular Gland.—No tubercles or tubercle bacilli have been found.

Prepectoral Gland.—The gland is highly vascular. There are no lesions or bacilli.

Right Precrural Gland.—No lesions or tubercle bacilli have been found.

Left Precrural Gland.—There are several small tubercles. Each consists merely of a few giant cells or cell fusions situated in a reticulum of diffusely staining material which is continuous with the processes of these cells.

Two tubercle bacilli have been found in these foci.

SUMMARY.

In one gland there are very slight and obviously retrogressive lesions; in the others no lesions or bacilli have been found.

VIRUS.—B IV.

Animal Fed.—Calf 188.

Calf 188 was fed with 1,000,000 bacilli of B IV., contained in milk, and was killed 44 days afterwards.

Mesenteric Gland.—There are large tubercles, which are in an advanced stage of caseation, and also smaller lesions. In the latter there is only a slight amount of caseation, giant cells are numerous, and there is a great increase of fibrous tissue.

Tubercle bacilli are scanty.

SUMMARY.

Tuberculosis of the chronic type with marked evidence of retrogressive change.

VIRUS.—B IX.

Animal Fed.—Calf 380.

Calf 380 was fed with 1 mg. of culture of B IX. and was killed 140 days afterwards.

Left Bronchial Gland.—The gland is very extensively caseous and contains numerous giant cells. There is considerable fibroid change.

Tubercle bacilli are present in all parts of the lesions, frequently within giant cells, but are not very numerous and never occur in groups.

SUMMARY.

Extensive caseous tubercles, of the more chronic type. It is noteworthy that these lesions were produced by feeding with a small dose of culture.

C.—LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 1. C.M.

Animal Inoculated.—Cow 3.

Cow 3 received an intramammary inoculation with an emulsion of bacilli, grown on potato, which had been isolated from a guinea-pig inoculated with the original material. Cow 3 was killed sixty-three days after inoculation.

The Left Iliac Gland.—Is in a condition of hyperplasia, but presents no lesions indicative of tubercular or any other definite disease histologically recognisable.

No tubercle bacilli have been found.

The Right Supramammary Gland.—Is oedematous, and contains caseous foci and giant cells, the latter measuring 30 to 45μ in their longest diameter.

A few bacilli have been found in various parts of the sections, the greater number of them within giant cells.

SUMMARY.

It appears that the infection has just succeeded in travelling to the nearest gland, and has there been arrested.

VIRUS.—H 1. C.M.

Animal Fed.—Calf 21.

Calf 21 was allowed to suck Cow 3 for 62 days. The udder of Cow 3 had been infected by intramammary inoculation. Calf 21 was killed 226 days after the commencement of the experiment.

Mesenteric Glands.—Two specimens have been examined. One appeared normal to the naked eye, the other contained some gritty foci and required decalcification. The former specimen shows no lesions under the microscope. In the latter specimen the lesions show very marked conservative change. Many of them consist merely of a mass of calcareous material surrounded by a dense wall of fibrous tissue. Small, non-calcareous lesions are also present. These are partially caseous in the centre and are surrounded by a zone of lymphocytes. A few giant cells occur. The lesions are small and isolated. The greater portion of the tissue is normal.

Several specimens have been carefully searched but only one tubercle bacillus has been found.

SUMMARY.

Isolated, retrogressive tubercles. Bacilli very rare.

VIRUS.—H 2. Sp. A.

Animal Fed.—Heifer 11.

Heifer 11 was fed with sputum for 7 months, and killed 209 days after the commencement of the experiment.

Mesenteric Glands.—Large, caseo-calcareous tubercles are present and there are also many smaller lesions which have not advanced to calcification. There is an increase of fibrous tissue at the periphery of many of the tubercles, but the lesions ramify irregularly in all directions and are not definitely circumscribed. Giant cells are numerous. Many of the blood-vessels show marked thickening of their outer coats

A considerable number of the giant cells contain from one to six tubercle bacilli. Bacilli are also present, though scanty, in the necrotic material. The bacilli vary in length from 2 to 4 μ; the majority of them are curved and have a tendency to beading.

SUMMARY.

Progressive tuberculosis of the chronic, caseo-calcareous type.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 79.

Calf 79 was inoculated subcutaneously with an emulsion of the tissues of Heifer 11, and was killed 71 days afterwards.

Sternal Gland.—The tissue is almost entirely replaced by caseous material which contains numerous bacilli.

Mediastinal Gland.—There is extensive necrosis with some fibroid change but no evidence that the process of tissue destruction is being arrested. There is some deposit of fibrin. Bacilli are numerous.

Hepatic Gland.—The tissue is riddled with caseating tubercles. Bacilli are fairly numerous.

SUMMARY.

Progressive tuberculosis. Caseation advanced. Bacilli numerous.

VIRUS.—H 2. Sp. A.

Animal Inoculated.—Calf 89.

Calf 89 was inoculated intravenously with an emulsion, estimated to contain 300,000 bacilli, from the tissues of Calf 79, and was killed, when very ill, twenty-three days after inoculation. No tuberculin test after inoculation.

Mediastinal Gland.—Advanced tubercular infiltration and necrosis, obliterating the greater part of the gland tissue. The tubercular areas are everywhere confluent. Tubercle bacilli are abundant and universally distributed.

Sections do not stain successfully by Pappenheim's method. The gland is swarming with ordinary multinuclear leucocytes. They are particularly abundant in the histologically tubercular areas. Though present everywhere, they are least numerous amongst some of the denser nodes of apparently normal lymphoid tissue There is a copious fibrinous exudate.

Bronchial Gland.—Similar in general appearance to the mediastinal gland.

As regards the distribution of bacilli and the copious infiltration of multinuclear leucocytes, this gland corresponds to the mediastinal.

SUMMARY.

Acute and rapidly progressive infection. Copious deposit of fibrin Bacilli abundant everywhere.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 5.

Calf 5 was inoculated subcutaneously with an emulsion of the original material, and was killed 109 days afterwards.

Bronchial Gland.—There is very advanced caseation, with numerous calcareous deposits. Less necrotic lesions are also present. These contain many giant cells and are surrounded by an abundant layer of fibrous tissue. Tubercle bacilli are scanty.

Summary.

Advanced, caseo-calcareous lesions with evidence of conservative change. Bacilli scanty.

VIRUS.—H 7. C.M.

Animal Inoculated.—Cow 73.

Cow 73 received an intramammary inoculation with an emulsion of the original material, and was killed 178 days later. Last tuberculin test, 16 days before death. Rise 1·3°C.

Supramammary Gland.—Large tubercles, chiefly in the cortex, generally bounded by fibrous tissue and exhibiting advanced caseation and calcification. Many giant cells. The specimens were decalcified by treatment for a few hours with sulphurous acid.

Tubercle bacilli are found, though not very numerous, in the caseous material, the calcareous deposits, and some of the giant cells.

Decalcified tissues do not as a rule stain well for finer histological details. This specimen has proved exceptional in that the oxyphil leucocytes are well brought out and differentiated from the other elements in eosin and methylene blue specimens. Oxyphil leucocytes are found abundantly throughout the peripheral zone of all the larger tubercles; in the interior of these tubercles they occur less regularly but are found not unfrequently, and still retaining an oxyphil stain, close up to the calcareous deposits. As regards other parts of the tissue, they occur in small and variable numbers in association with patches of giant cell formation; there are a few aggregations of them in parts of the cortex not definitely tubercular, and occasional aggregations in non-tubercular parts of the medulla.

Summary.

Caseo-calcareous tubercles, surrounded by a broad fibrous zone. Bacilli present, but not numerous.

VIRUS.—H 7. C.M.

Animal Inoculated.—Calf 101.

Calf 101 was inoculated intravenously with an emulsion, estimated to contain 48,000 bacilli, of the tissues of Calf 5, and was killed, when moribund, 41 days afterwards. No tuberculin test was made after inoculation. The following glands have been selected as specimens of early lesions.

Iliac Gland.—The greater part of the section appears normal, but at one place there are some small foci of commencing necrosis and several large giant cells.

Bacilli are numerous in these foci, and amongst and within the cells in their immediate neighbourhood. The cells which contain bacilli within their substance have the characters of endothelial cells and of lymphocytes; none of them are multinuclear. From about four to twenty bacilli are seen in each of the giant cells examined. Near, but apart from these two situations, small groups of bacilli are found which have produced very little histological change, the only noticeable feature being an occasional grouping of cell nuclei suggestive of commencing giant cell formation. Multinuclear cells are not noticeable in these areas. In the greater portion of the section no bacilli are found either in the lymphoid tissue or in the lymph sinuses.

In the non-affected portions of the gland the lymphocytes are present in normal numbers and react well to differential stains. Small coarse oxyphils are not numerous; they are found in the midst of the lymphoid tissue; they do not form aggregations either in the capsule or in the fibrous trabeculæ. Finely granular multinuclears are also present but are rare.

In the infected areas the most conspicuous feature in Pappenheim specimens is the entire absence of a red protoplasmic stain from the foci of commencing necrosis. The giant cells vary in their staining reaction by this method. Some of them only take up the green nuclear stain; others have a faint pinkish protoplasmic tinge; and a few are in parts definitely red. Apart from these two situations the red protoplasmic lymphocytes are numerous and stain well; the number in mitotic division is little, if any, more than normal; occasionally a few of them adhere together more closely than is usual and offer some resemblance to commencing giant cell formation. In these specimens characteristic lymphocytes cannot be demonstrated as taking an active part in the formation of the tubercular foci, and are only in a few instances definitely associated with the giant cells. There is no diminution, in the infected part of the gland, in the number of coarsely granular oxyphils, though these cells do not occur actually within the definitely tubercular foci. Finely granular leucocytes are only slightly more numerous in the latter situations than in the non-infected parts; one or two may be found within or near most of the areas of commencing necrosis; there is nowhere any indication of a leucocytic infiltration. There is nothing abnormal in the vascularity of the affected part. Within and in proximity to the affected sites there is some evidence of a proliferation of endothelial cells. In most of the necrosing areas nuclei characteristic of this type of cell survive and appear in larger numbers than in normal lymphoid tissue. Most of the giant cells appear to be of endothelial origin. In addition to definite giant cells, there are irregular groups of cell nuclei surrounded by vaguely defined, branching protoplasm which takes a pinkish tinge in Pappenheim specimens; the colour of the protoplasmic stain, and the characters of the individual nuclei in these groups closely correspond to the appearances of endothelial cells in normal glands. An endothelial cell never takes a bright red protoplasmic stain like a lymphocyte. There is no evidence of the formation of fibrous or granulation tissue round the tubercular foci. A fibrinous deposit is found in almost every situation where tubercle bacilli occur, except when they occur within giant cells, but is not found apart from bacilli.

Bronchial Gland.—Small foci of commencing necrosis and giant cells. Lesions more generally distributed than in iliac gland.

The distribution of bacilli is the same as in the iliac gland; none have been noticed within multinuclear leucocytes.

The gland contains a large number of lymphocytes the protoplasm of which stains a deep red by Pappenheim's method. Many of them are undergoing mitotic division, but the number of these is not greater near the tubercular areas. The relationship of the lymphocytes to the infected areas is the same as in the iliac gland. A few of the giant cells exhibit a patch of material which stains deeply red at one part of their periphery. It is impossible to interpret this appearance with any certainty. It may indicate that the cells in question were formed from lymphocytes, or it is possible that, while in process of formation from endothelial cells, a few lymphocytes became entangled amongst their nuclei. Coarsely granular eosinophils occur in small numbers in the gland substance. Throughout the medullary regions and in all the lymph sinuses finely granular oxyphil leucocytes of the ordinary polymorphonuclear types occur in numbers far above the normal, though not in such large numbers as in the right prescapular gland of Calf 135. A few multinuclear oxyphils, as a rule not coarsely granular, are found in the tubercular foci, but there is no definite determination of these cells to these areas; much the greater number of them are found in the lymph sinuses. The vascularity of the gland is increased, and there are many red blood corpuscles lying free in the lymph sinuses. In the proliferation of endothelial cells, the absence of new fibrous or of granulation tissue, and the presence of fibrin in association with the bacilli, this gland resembles the iliac gland.

Coeliac Gland.—Lesions of the same type as in the two previous glands, but more advanced.

Distribution of bacilli, lymphocytes, and coarse eosinophils, the same. Finely granular oxyphils rare. Vascularity not increased. Proliferation of endothelium more pronounced than in the two former glands and proliferation of capillary endothelium also noted. No fibrous or granulation tissue round tubercular foci, and no alteration in the appearance of the lymphocytes immediately external to these foci. Fibrinous exudate more copious than in the two former glands.

Summary.

These specimens, selected in the post-mortem room with the object of studying early lesions, illustrate very well the rapidity of the infective process in calves inoculated intravenously with a small dose of a virulent human virus.

VIRUS.—H 7. C.M.
Animal Inoculated.—Calf 105.

Calf 105 was inoculated subcutaneously with an emulsion from the tissues of Calf 5, estimated to contain 48,000 bacilli, and was killed, when moribund, 47 days afterwards.

Popliteal Gland.—There are several caseous tubercles. These are of irregular outline and are not surrounded by any definite cellular zone. No fibrin is demonstrable.

Tubercle bacilli occur in moderate numbers.

Summary.

Progressive tuberculosis.

VIRUS.—H 7. C.M.
Animal Inoculated.—Calf 109.

Calf 109 received an intraperitoneal inoculation with an emulsion from the tissues of Calf 5, estimated to contain 48,000 bacilli, and died 40 days afterwards.

Bronchial Gland.—There are extensive lesions which are in a condition of early caseation. No giant cells are present and there is no sign of any boundary zone at the periphery of the tubercles.

Tubercle bacilli are very numerous.

Summary.

Acute, progressive tuberculosis. Bacilli numerous.

VIRUS.—H 7. C.M.
Animal Inoculated.—Calf 141.

Calf 141 was inoculated subcutaneously with an emulsion estimated to contain 250,000 bacilli, from the tissues of Cow 73, and was killed 53 days afterwards. No tuberculin test was made after inoculation.

Hepatic Gland.—Advanced caseo-calcareous lesions more or less continuous with one another and permeating the greater part of the section.

Bacilli are fairly numerous in all the parts histologically tubercular, both in the caseous areas and amongst the cells which show no signs of breaking down.

In the non-tubercular parts of the gland many of the large lymphocytes show mitotic figures. In the strands of tissue which have survived between the caseous areas it is difficult to say how many of the cells which react to Pappenheim's stain are normal lymphocytes and how many are plasma cells; cells which cannot be distinguished from lymphocytes and all varieties of plasma cells are present. Oxyphil leucocytes occur in somewhat small numbers; there is no marked leucocytic infiltration of the necrosing areas. External to the caseous tracts there is a tendency to fibrous tissue formation, giving way to necrosis. Both old and recent deposits of fibrin are found.

Summary.

Advanced, progressive caseo-calcareous lesions. Bacilli numerous. Fibrin present.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 267.

Calf 267 was inoculated subcutaneously on the right side of the neck with an emulsion of pure culture of original material killed by heating. Calf 267 was killed 111 days afterwards.

Right Prescapular Gland.—Two portions of the gland have been examined. In neither have either tubercles or tubercle bacilli been found.

SUMMARY.

The absence of any lesions in the gland draining the seat of inoculation illustrates the wide difference between dead bacilli and bacilli which, when living, are of only slight virulence for the bovine.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 305.

Calf 305 was inoculated intravenously with an emulsion estimated to contain over 700,000,000 bacilli of the organs of guinea-pigs which had been inoculated from Calf 177. Calf 305 was killed, when very ill, 26 days after inoculation. No tuberculin test was made after inoculation.

Right Precrural Gland.—A few small, suspiciously pale areas are visible in the cortex.

All these areas contain numerous bacilli. Bacilli are also scattered freely throughout the rest of the cortical lymph nodes, but none are found in the medullary part of the gland. Bacilli are very often contained within the endothelial cells which are found within the cortical lymphoid tissue and lining the lymph sinuses adjacent to this tissue. The bacilli are long and curved, closely resembling those of the emulsion with which the calf was inoculated.

The large lymphocytes are normal in the medullary part of the gland, but in the cortex there is a diminution of their numbers; their absence is noted in the places where bacilli are present; the diminution, in the cortex, of the small lymphocytes is relatively less. No coarse eosinophils have been observed. Ordinary multinuclear leucocytes are not abundant, but are in excess of the normal. They are found in small numbers in all the lymph sinuses and amongst most of the tubercular foci; they are also noticeable in the blood capillaries in proximity to these foci. The gland is well supplied with blood-vessels, but they are not dilated, and there is no noticeable extravasation of red corpuscles. There is a well-marked increase of endothelial cells in the cortex, particularly in the tubercular foci. There are no giant cells. No fibrin is present.

Right Popliteal Gland.—Similar to right precrural.

Distribution and characters of bacilli and of lymphocytes the same as above; a few coarse eosinophils are present. Finely granular oxyphils, vascularity, and endothelial cells as in precrural gland. In a few places there is a slight fibrinous exudate.

Bronchial Gland.—The cortex is more extensively affected than in the two previous glands, and patches of commencing necrosis are also seen in the interior of the gland.

Bacilli are plentiful, and their distribution is more extensive than in the two former cases, corresponding to the greater extent of the histological lesions.

The characters of the bacilli and their relations to cells are the same as before.

In the medullary part of the gland large lymphocytes are very abundant, and frequently show mitotic figures. In a few small lymphoid nodes which are of the cortical type (*i.e.*, contain very large numbers of small lymphocytes), and are not tubercular, the number of large lymphocytes is small. In the cortex, although it is extensively infected with bacilli, the number of cells which resemble large lymphocytes in their reaction to Pappenheim's stain is very considerable. These cells are aggregated in groups which surround all the foci of commencing tubercular necrosis. On examination with a higher power, it is found that while many of these cells cannot be distinguished morphologically from ordinary hyaline lymphocytes, every grade of transition is present between this type of cell, the plasma cell with a flattened rectangular outline, and the plasma cell assuming the spindle shape of a fibroblast. The cell groups as a whole are identical with areas of infiltration by plasma cells, which are found in tubercular tissues other than lymphatic glands. The inference indicated by these observations, taken in conjunction with the fact that in earlier stages of lymphatic infections the large lymphocytes normally present in the cortex rapidly disappear from the tubercular foci, is that the cells in question are plasma cells which have migrated into the cortex from the medulla, where they have been reproduced from normal lymphocytes.

Coarse eosinophils are present in small numbers. Finely granular oxyphils are numerous everywhere. Their numbers are greatest in the lymph sinuses in the interior part of the gland: in the cortex they occur most abundantly in the tubercular foci; within intact blood-vessels their numbers are greater than normal. There is a considerable extravasation of red blood corpuscles, chiefly in the sinuses found in the interior part of the gland. The increase of endothelial cells in the tubercular areas is similar to that found in the two previous glands, but more extensive. No giant cells have been noticed. There is a well-marked deposit of fibrin wherever bacilli are numerous.

Posterior Mediastinal Gland.—Very similar to the bronchial gland, but slightly more advanced.

The characters and distribution of bacilli are the same as in the last specimen; the bacilli are much more numerous in this gland, especially in the advanced areas.

As regards the lymphocytes and the abundant infiltration of the tubercular areas with typical plasma cells, this gland is identical with the last. Coarse eosinophils are found occasionally, even in the tubercular areas. Finely granular oxyphils are even more abundant than in the last specimen, especially in the internal lymph sinuses. In the more advanced lesions, many of them have lost their differential protoplasmic stain, but their characteristic nuclei are preserved and are found in numbers which show that the infiltration of these leucocytes has been very extensive. Multinuclear leucocytes are also found in abnormally large numbers within the blood-vessels. The number of red blood corpuscles lying free in the lymph sinuses is as great as in the last-described gland. As in the last specimen, a ground substance of endothelial cells is found in all the tubercular areas. Fibrin is abundant and fairly recent.

SUMMARY.

Minute lesions, but numerous and apparently progressive. Bacilli abundant everywhere. Fibrin present.

VIRUS.—H 8. S.C.

Animal Inoculated.—Calf 361.

Calf 361 was inoculated intravenously with an emulsion, estimated to contain 41,500,000 bacilli, from the tissues of Calf 275, and was killed 76 days afterwards.

Peribronchial Gland.—The gland is very extensively caseous and contains many giant cells.

Tubercle bacilli are distributed throughout the lesions in small numbers and occasionally occur in small groups.

SUMMARY.

Extensive caseation; many giant cells; tubercle bacilli present in all the lesions, but not abundant.

VIRUS.—H 9. C.T.

Animal Inoculated.—Calf 185.

Calf 185 was killed 40 days after subcutaneous inoculation with ·75 cc. of bacilli from an old broth culture. No tuberculin test was made after inoculation.

Left Prescapular Gland.—The section consists of caseo-calcareous material interspersed with strands of cellular substance; these are mostly fibrous, but contain small islands of lymphoid tissue. Giant cells are numerous.

In the necrotic areas bacilli are found in moderate numbers; they are also present both within and in association with giant cells. Very few occur amongst the other cellular elements.

In the small patches of lymphoid tissue which have escaped destruction the lymphocytes are nearly all of the small type. No coarse eosinophils are to be found. Ordinary oxyphil leucocytes with a fine or obscure granulation are abundantly scattered throughout the parts of the tissue which retain a cellular stain. These areas are also highly vascular. The characters of the nuclei and protoplasm of the giant cells bear no evidence of relationship to either lymphocytes or plasma cells. Typical chains of plasma cells passing into fibroblasts are numerous. There is old but no recent fibrin.

SUMMARY.

Chronic lesions containing moderate numbers of bacilli.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 301.

Calf 301 was inoculated subcutaneously with an emulsion, estimated to contain over 540,000,000 bacilli, of the tissues of guinea-pigs inoculated from Calf 129. Calf 301 was killed, when very ill, 33 days after inoculation. No tuberculin test was made after inoculation.

Left Popliteal Gland.—Extensive foci of fairly advanced necrosis in the cortex, and also much smaller and earlier patches. Many giant cells. Medulla only slightly affected.

Colonies of bacilli can be seen in the cortex with a low power. Throughout the affected regions they are present in enormous numbers; the giant cells always contain some, and generally a large number. I have found a giant cell which answers to the description of these bodies met with in text-books; the nuclei are all arranged peripherally and regularly, forming a complete circle, immediately internal to which is a circle of bacilli, whilst the central part of the cell is unoccupied. The bacilli are found only in definite areas, all of which exhibit histological change. More than half of the total area of the section examined is free from bacilli. It is unusual in a bovine gland containing bacilli in enormous numbers to find them so strictly localised. The bacilli are noticeably shorter and straighter than those in the emulsion with which the animal was inoculated.

As in the more advanced of the specimens taken from Calf 305, most of the tubercular patches are partially surrounded by cells which appear to represent an infiltration of plasma cells. No coarse eosinophils have been found. Finely granular oxyphils form dense aggregations in the centre of many of the larger tubercular areas, and are conspicuous and generally numerous within and in association with nearly all the foci which are histologically tubercular. They are fairly numerous in the lymph sinuses and markedly above the normal within blood vessels. The blood vessels are not well filled; the number of red corpuscles lying free amongst the tissue is not great. Cells of endothelial type have greatly increased in numbers and form the groundwork of the tubercular areas. There is no noticeable increase of fibrous tissue which stains differentially by van Gieson's method. Fibrin is present.

Deep Cervical Gland.—Similar to the last, but the lesions, though not more advanced, individually, are more generally distributed throughout the gland.

Bacilli are found everywhere, and in the older lesions are as abundant as in the previous gland. They are also similar in their characters and in their relationship to giant cells, which are again numerous.

In Pappenheim specimens the entire section is occupied by pale, more or less circular areas which are surrounded by a zone of cells with red protoplasm. Whilst some of these cells are indistinguishable in appearance from lymphocytes, the greater number of them resemble typical plasma cells. Finely granular oxyphil leucocytes are very abundant throughout the tissue; in the older lesions many of them have lost their red protoplasm. Blood capillaries are to be found in every part of the gland. The distribution of endothelial cells is similar to that in the popliteal glands. Van Gieson specimens show a greater increase of fibrous tissue surrounding the tubercles than in the popliteal gland. Fibrin is present, but not recent.

SUMMARY.

Numerous progressive tubercles. Giant cells present. Some fibrin. Bacilli very abundant. In proportion to their numbers, the amount of tissue destruction is relatively small.

VIRUS.—H 13. A.D.

Animal Inoculated.—Calf 325.

Calf 325 was inoculated subcutaneously with an emulsion from the tissues of Calf 301, containing approximately 500,000,000 bacilli. Calf 325 was killed, when moribund, 24 days afterwards.

Bronchial Gland.—The gland structure is almost entirely replaced by caseating material which is not limited to definite tubercles and shows no sign of conservative change. There is a coagulated deposit which is probably old fibrin; no recent fibrin is demonstrable.

Tubercle bacilli are extremely numerous; they are aggregated into groups which form conspicuous objects under a low power.

SUMMARY.

Acute tuberculosis. Bacilli very numerous.

VIRUS.—H 14. F.S.

Animal Inoculated.—Calf 121.

Calf 121 was inoculated subcutaneously with an emulsion of the original material estimated to contain over 360,000,000 bacilli. The animal was killed, when ill, 36 days afterwards.

Popliteal Gland.—There are numerous tubercles in an advanced stage of caseation. The lesions ramify in all directions and show no signs of a limitation zone. No giant cells are found.

Tubercle bacilli are abundant and are notably short and straight.

SUMMARY.

Acute tuberculosis.

VIRUS.—H 14. F.S.

Animal Inoculated.—Heifer 243.

Heifer 243 was inoculated subcutaneously with an emulsion, estimated to contain 4,500,000 bacilli, from the tissues of Heifer 197, and was killed, when very ill, 61 days afterwards. A tuberculin test, made five days before death gave a rise of 1·1° C.

Hepatic Gland.—Extensive but discrete tubercles in an advanced stage of caseation, and calcareous centrally. Minute foci in an earlier stage, interspersed between these.

Tubercle bacilli are very scarce, both in the larger and smaller lesions.

Both in the non-tubercular parts of the gland and quite close to the lesions, coarse eosinophils are found in small numbers. Ordinary multinuclear leucocytes nowhere form conspicuous aggregations. At the margins of some of the caseous areas, small groups of them are to be seen, but in several of these situations they are absent. They occur rather frequently entangled in the fibrous stroma at the periphery of the tubercles; in this situation they tend to become coarsely granular. In the rest of the tissue they are rare. There is a definite proliferation of endothelial cells at the margin of the tubercles; some of these cells are in continuity with the walls of blood capillaries. In these situations, cells are also present which have the appearance and staining reactions of plasma cells passing into fibroblasts. There is an increase of adult fibrous tissue, chiefly at the periphery of the tubercles, but growing irregularly and not forming nodules which demarcate the tubercular areas.

SUMMARY.

Large, discrete, caseo-calcareous tubercles; also earlier lesions. Vigorous tissue resistance. Bacilli very rare.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 273.

Calf 273 was inoculated subcutaneously with an emulsion from the tissues of Calf 187 containing, approximately 11,000,000 bacilli. Calf 273 was killed 89 days afterwards.

Thoracic Gland.—Many tubercles, of various sizes, are present. The largest are in an advanced stage of caseation and contain a little calcareous deposit. The smaller are only slightly caseous. All the tubercles contain many giant cells. They exhibit some fibrous tissue reaction at their periphery, but the layer of fibroblasts is nowhere dense and the tubercles are evidently extending.

Bacilli are present in small numbers in most of the tubercles.

SUMMARY.

Caseous tubercles of the slowly progressive type are present. Bacilli are not numerous.

VIRUS.—H 16. J.H.

Animal Inoculated.—Calf 281.

Calf 281 was inoculated subcutaneously with an emulsion of the tissues of Calf 355 containing, approximately, 11,000,000 bacilli. Calf 281 was killed when ill, 54 days afterwards.

Right Axillary Gland.—Throughout the gland there is a network of semi-caseous patches. No giant cells are present and there is no demarcation layer surrounding the patches. The affected areas are infiltrated with multinuclear leucocytes.

Tubercle bacilli are moderately numerous.

SUMMARY.

Extensive and progressive tubercular infiltration.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 529.

Calf 529 was inoculated intravenously with an emulsion from the tissues of Calf 539 containing, approximately 1,000,000,000 bacilli. Calf 529 died 13 days afterwards.

Sternal Gland.—The blood-vessels are highly congested. No tubercles or other histologically recognisable lesions are present.

Tubercle bacilli are present in many parts of the section and often occur in small groups. They are very frequently intracellular.

SUMMARY.

The gland is congested. Tubercle bacilli are present but have produced no formation of tubercles.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 555.

Calf 555 was inoculated subcutaneously with an emulsion from the tissues of Calf 553 containing, approximately, 150,000,000 bacilli. Calf 555 was killed, when moribund, 58 days afterwards.

Lumbar Gland.—The gland shows diffuse caseation without any demarcation into definite tubercles. No giant cells are present and there is no formation of new fibrous tissue. There is an abundant deposit of fibrin.

Tubercle bacilli are very abundant.

SUMMARY.

Acutely progressive tuberculosis. Bacilli abundant.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Calf 685.

Calf 685 was inoculated intravenously with 10 mg. of culture isolated from Calf 339, and killed, when in good health, 64 days after inoculation.

Bronchial Gland.—The specimen is histologically normal.

One tubercle bacillus has been found with difficulty.

SUMMARY.

No lesions have been produced and only one bacillus has been found.

VIRUS.—H 18. T.T.

Animal Inoculated.—Calf 405.

Calf 405 was inoculated subcutaneously with an emulsion from the organs of Guinea-pig 173, containing about 270,000,000 bacilli, which was one of a series inoculated from Calf 131. Calf 405 was killed 55 days afterwards.

Bronchial Gland.—Immediately beneath the capsule there are two minute foci of early caseation. The rest of the tissue appears normal, with the exception of a few very minute points; some of these apparently represent the partial necrosis of about half-a-dozen cells; in others again there are a few fragments of cell nuclei, and cells of the fibroblast type are more conspicuous than normal.

Each of the caseous foci mentioned above contains several bacilli which are notably long and curved. In two of the suspicious points present in the rest of the tissue a tubercle bacillus has been found.

SUMMARY.

Very slight tuberculosis.

VIRUS.—H 19. S.W

Animal Inoculated.—Calf 271.

Calf 271 was inoculated subcutaneously with an emulsion, estimated to contain nearly 16,000,000 bacilli, from the tissues of Calf 179, and was killed, when very ill, 36 days subsequently. No tuberculin test made after inoculation.

Supramammary Gland.—One or two small patches of commencing tissue necrosis.

Bacilli are found in small numbers in these areas, and a few small groups of bacilli occur elsewhere, where there is no obvious change.

Very few coarsely granular eosinophils are present. There is a definite and moderately great increase in the finely granular oxyphils in the lymph sinuses, in the lymphoid tissue, and within the blood-vessels. The rather more advanced of the tubercular foci also contain these cells in fairly large numbers. The blood vessels are not abnormally dilated, and there is no extravasation of red corpuscles. There is a proliferation of endothelial cells in the infected areas, but there are no giant cells. There is no tendency to a demarcation of the infected areas by a formation of fibroblasts. In Kochel specimens the bacilli are found to be everywhere associated with a deposit of fibrin. This is true not only of the larger foci but also of the very small groups of bacilli, which, in specimens stained by other methods appear to have produced little or no tissue alteration.

Lumbar Gland.—Similar to the supramammary.

Many lymphocytes show mitotic figures. A few coarse eosinophils are found. The gland is swarming with finely granular multinuclear leucocytes, which are universally distributed. The medullary lymphoid tissue contains them in relatively largest amount, but they are also very numerous in all the lymph sinuses, and in the cortical lymph nodules. They are abundant in tubercular foci where necrosis is commencing, but, considering their abundance in the non-tubercular parts of the gland, there appears to be no special determination of them to the tubercular foci. The intact blood vessels are not well filled, but the number of leucocytes within them appears to be in excess of the normal. Red blood corpuscles are found in considerable numbers lying free in the lymph sinuses and amongst the lymphoid tissue. With regard to the increase of endothelial cells in the tubercular areas and the association of fibrin with the bacilli, this gland corresponds to the supramammary.

SUMMARY.

Small, progressive lesions. Bacilli not numerous. Fibrin present.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 399.

Calf 399 was inoculated intravenously with an emulsion of the lesions of Guinea-pig 200, containing over 700,000,000 bacilli, which was one of a series inoculated from Calf 291. Calf 399 was killed when very ill, 34 days afterwards.

Bronchial Gland.—The entire section is mottled with pale areas in which the lymph cells, though not obliterated, stain faintly and show partial necrosis and fusion of their protoplasm. In these areas cells with elongated, faintly stained nuclei, probably derived from the glandular reticulum, are very numerous. Giant cells are occasionally found. There is an abundant infiltration with multinuclear leucocytes. No fibrin is present.

All these areas of commencing necrotic change are due to the presence of large numbers of tubercle bacilli. A great many of the bacilli are intracellular.

SUMMARY.

The gland is densely infiltrated with tubercle bacilli which have set up early necrotic change but, in proportion to their numbers, have caused a relatively small amount of tissue destruction.

VIRUS.—H 22. F.W.

Animal Inoculated.—Calf 749.

Calf 749 was inoculated intravenously with 46 mg. of culture isolated from Calf 293, and was killed when very ill, 24 days afterwards.

Bronchial Gland.—The protoplasm of the lymph cells is not sharply defined, and the gland contains an abnormal number of multinuclear leucocytes. There are no tubercles or definitely necrotic areas. No fibrin is present.

The gland is swarming with tubercle bacilli which have multiplied into groups throughout the cortical portion. Most of the bacilli are long and curved.

Mesenteric Gland.—The condition is similar on the whole to that of the bronchial gland, but bacilli are rather less numerous.

SUMMARY.

Enormous numbers of bacilli are present but there are no histological tubercles and the amount of tissue damage is only slight.

VIRUS.—H 23. J.P.

Animal Inoculated.—Calf 365.

Calf 365 was inoculated subcutaneously with an emulsion, estimated to contain over 100,000,000 bacilli, of the original material, and was killed 61 days afterwards.

Right Prescapular Gland.—The gland is very extensively necrotic and caseous, the surviving tissue being of a densely fibroid character and containing some giant cells. Most parts of the section, including caseous areas, contain very few bacilli. In a few of the caseous patches, however, tubercle bacilli are extremely numerous.

Histologically, the parts which retain a cellular stain exhibit the usual characters of old and advanced lesions, viz., infiltration with oxyphil leucocytes, copious plasma cell and fibrous tissue formation, and evidence of endothelial proliferation.

Mediastinal Gland.—There are many small, slightly caseous tubercles. Giant cells are plentiful both within these tubercles and as isolated structures amongst the lymphoid tissue.

Tubercle bacilli are rare.

No fibrin is present.

The gland is highly vascular and contains a large number of multinuclear leucocytes, which exhibit a definite determination towards the tubercular foci.

Mesenteric Gland.—Compared with the mediastinal gland examined, some of the areas of caseation are larger and there is no condition of hyperæmia.

In a few of these larger caseous areas tubercle bacilli are rather plentiful.

With the exceptions mentioned, the gland is similar to the mediastinal.

SUMMARY.

The prescapular gland contains numerous bacilli. The mediastinal and mesenteric glands contain small caseous tubercles in which giant cells are plentiful, but bacilli, with the exception of a few areas, scanty. These last mentioned glands do not contain any fibrin.

VIRUS.—H 25. A.T.
Animal Inoculated.—Calf 551.

Calf 551 (a bull-calf 10 months old) was inoculated intravenously with 100 mg. of culture isolated from Calf 417, and died 21 days afterwards.

Thoracic Gland.—Lesions are present in the specimen, but are few and minute, consisting of nothing more than groups of cells which stain imperfectly and have lost the sharpness of their protoplasmic outline but have not formed definite tubercles.

Bacilli are present in small numbers, and are generally attached to endothelial cells covering the lymph spaces.

SUMMARY.

Bacilli are present in small numbers but have caused hardly any tissue change.

VIRUS.—H 25. A.T.
Animal Inoculated.—Calf 831.

Calf 831 was inoculated intravenously with an emulsion of the tissues of Calf 551, containing about 1,000,000,000 bacilli, and died 17 days afterwards.

Iliac Gland.—There are numerous minute foci which consist of a fused mass of protoplasm of irregular outline. In this material cell nuclei are distributed sometimes quite irregularly and sometimes in the form of a more or less complete circle. In the latter case the appearance is that of a giant cell. Most of the nuclei in these areas are large, oval, and stain lightly; together with these, the spherical deeply-staining nuclei of small lymphocytes are often present.

In all the foci referred to, small groups of tubercle bacilli are present; bacilli are also frequent in the rest of the tissue, generally occurring singly or in pairs and usually within cells

SUMMARY.

Bacilli are numerous and have provoked a reaction characterised by the presence of many giant cells but have not led to the formation of organised tubercles.

VIRUS.—H 29. M.F.
Animal Inoculated.—Calf 477.

Calf 477 was inoculated subcutaneously with an emulsion of the organs of guinea-pigs which had been inoculated with the original material. The dose was estimated to contain about 3,000,000,000 bacilli. Calf 477 was killed, when moribund, 34 days afterwards.

Popliteal Gland.—There are numerous caseating tubercles which show no fibrous limitation, but contain an abundant deposit of fibrin. The lesions are typical of the acute form of the disease.

Tubercle bacilli are present in large numbers.

SUMMARY.

Acute, progressive tuberculosis.

ANALYSIS OF RESULTS.

I.

LESIONS OF THE UDDER.

VIRUS.—B I.

Cow 18.—This is an example of highly acute infection with rapidly progressive tissue destruction and great multiplication of bacilli. This result is in accordance with the high degree of virulence established for this virus by the 50 mg. culture inoculations. The dose inoculated into the udder is not stated, but as it was obtained from the organs of guinea-pigs, where tubercle bacilli multiply rapidly, it was probably large.

Cow 40.—The lesions produced are of a chronic, slowly progressive type. They are densely fibrous and calcareous and contain many giant cells, whilst bacilli are extremely scanty. The disease, therefore, is notably different from that produced, by the same virus, in the udder of Cow 18. In explanation of this difference it is to be noted that Cow 40 was inoculated with a primary culture, 57 days old. Culture bacilli, even when in a condition of maximum vigour, are always less effective than an equal number of bacilli, belonging to the same strain, which have been grown in animal tissues. It is also to be noted that primary cultures grow less abundantly than subcultures, and that a culture 57 days old has exceeded the age of maximum vigour.

Cows 44 *and* 64.—In both udders there was produced a progressive caseating tuberculosis which calls for no special comment.

Cow 74.—This animal survived the inoculation for 299 days. Many animals have either died or been killed when moribund at a very much shorter period after receiving an intramammary inoculation. The virulence of the material inoculated was therefore in this case relatively low. The special histological features of the udder are that bacilli are present in enormous numbers, that the amount of tissue destruction which they have produced is relatively small, and that a great many of the bacilli are intracellular and appear to be multiplying within cells. The capacity of the bacilli for multiplication has been greater than their capacity for tissue destruction. The disease produced, therefore, presents a marked difference from the more acute type of lesions found in the udder of Cow 18. It is also to be contrasted with the udder of Cow 40, which is of the fibro-calcareous type, and contains very few bacilli. The special interest of the latter contrast lies in the fact that the material with which Cow 74 was inoculated was an emulsion of the mammary tissue of Cow 40.

VIRUS.—B II.

Cows 4 *and* 500.—The acute infection produced is consistent with the virulent character of this virus as established by the 50 mg. culture inoculations.

VIRUS.—B III.

Cow 68.—Advanced and progressive infection has been produced, but bacilli are less numerous, and there is more evidence of tissue reaction than in the very acute cases.

VIRUS.—B IV.

Cow 172.—This animal was killed 392 days after inoculation. The lesions in the udder are of the chronic, slowly progressive type, and contain few bacilli. As the virus used has been shown by a large number of other experiments to be of high virulence, the mildness of the infection produced in this animal is noteworthy. The dose (14,500,000 bacilli into each of two quarters) was large, but perhaps many of the bacilli were either dead or in a condition of feeble activity. They were obtained in an emulsion of the local lesion and prescapular gland of Calf 138. This animal was killed 202 days after inoculation. The local lesion, according to the *post-mortem* notes, was "small and fibroid," and the prescapular gland was "slightly enlarged and beset with calcareo-caseous masses."

VIRUS.—H 1. C.M.

Cow 3.—The disease is of the chronic, slowly progressive type, and is similar to that produced in the udder of Cow 172. The differences in the nature and source of the material used for inoculation in the two cases are of interest. Cow 3 was inoculated with a potato culture of a bacillus isolated from human sputum; this virus has been shown by a large number of experiments to be of low virulence. Cow 172 was inoculated with a tissue emulsion containing bacilli derived from a bovine virus of high virulence.

VIRUS.—H 7. C.M.

Cow 73.—This animal was inoculated with an unknown dose of a virus which, from other experiments, has been shown to be of high virulence. At frequent intervals after inoculation samples of the milk were inoculated into guinea-pigs, with the result that general tuberculosis was produced. The histological examination reveals no definite evidence of tuberculosis but shows that there has been a mastitis, possibly set up by the tubercle bacilli, which is subsiding. The bacilli which infected the guinea-pigs may have lodged and multiplied in the milk ducts and sinuses.

VIRUS.—H 10. B.S.

Heifer 249.—The interest of this case lies in the fact that slight tuberculosis of the udder developed 54 days after subcutaneous inoculation in the neck with a large dose of virulent virus.

Cow 295.—This animal received an intramammary inoculation with a moderately large dose (2,000,000 bacilli into each of two quarters) of the same virus, but only a small amount of disease was produced. The animal died suddenly, shortly after parturition and 179 days after the inoculation had been made. Possibly pregnancy may have had a retarding influence on the progress of infection.

VIRUS.—H 14. F.S.

Cow 75.—This animal was killed, when very ill, 34 days after intramammary inoculation and exhibited acute tuberculosis of the udder. The disease, in this case produced by a virus of human origin, is similar to the disease produced by some of the bovine viruses. The morbid process appears rather more acute than in the udder of Cow 68 and perhaps not quite so acute as in the udders of Cows 4, 18, and 500.

VIRUS.—H 19. S.W.

Heifer 233.—Into this case many complicating factors enter. The animal, older and therefore probably more resistent than the bovines usually employed, was inoculated subcutaneously in the neck with a large dose (over 50,000,000 bacilli obtained from the tissues of Calf 271). The virulence of these bacilli is proved by the fact that they produced very severe disease in Calf 271. Heifer 233 was pregnant when inoculated; this condition is generally considered to have a retarding influence on the progress of tuberculosis. The animal gave birth to a calf 54 days after inoculation and was killed 181 days after inoculation. After parturition tuberculosis, when present, is generally found to advance with increased rapidity. The udder of this animal shows a slight degree of tuberculosis. Possibly this condition supervened upon a non-tubercular mastitis following parturition. The condition of the lesions offers an interesting resemblance to some cases of human mammary tuberculosis.

Heifer 239.—This is a case of slight tuberculosis of the udder following subcutaneous inoculation in the neck with a dose of about 1,000,000 bacilli. The animal was killed 177 days after inoculation. This is an instance where tuberculosis of the udder was present, though it probably could not have been diagnosed during life. Even at the post-mortem examination, the diagnosis was not established. The following is the full description, taken from the post mortem notes:—"*Mammary Gland.*—Weighs 5 lbs. 6 ozs. when freed from skin and fat. All four quarters are much enlarged, and on section are found to be composed of typical functional mammary tissue. The ducts and sinuses are full of a turbid semitranslucent yellow fluid, resembling melted butter, and containing numerous little opaque specks."

Calf 597.—This was a young animal which was inoculated subcutaneously with 50 mg. of culture, the result being that tuberculosis was rapidly disseminated all over the body. The imperfectly developed mammary gland is in a condition of early, progressive tuberculosis.

VIRUS.—H 28. C.L.

Cow 143.—Acute, rapidly progressive tuberculosis of the udder was produced, similar in general histological characters to the disease caused by the injection of bacilli of bovine origin. The dose inoculated was large (20,000,000 bacilli into each of two quarters). The result of the inoculation is in accordance with the high virulence of this virus as established by the 50 mg. culture inoculations.

VIRUS.—H 29. M.F.

Heifer 251.—This is another case where the complication of pregnancy arises. The animal was inoculated subcutaneously with a large dose (about 3,000,000,000) of bacilli contained in the tissues of Calf 479. These bacilli had proved to be of high virulence for Calf 479. Heifer 251 remained in apparently good health and was killed after the lapse of 138 days. Nine days before the termination of the experiment the heifer gave birth to a calf. The udder is in a condition of early and apparently progressive tuberculosis.

Calf 477.—This animal was inoculated subcutaneously with a dose estimated to contain the same number of bacilli as the dose given to Heifer 251. Calf 477 was killed, when moribund, 34 days after inoculation. The mammary tissue is in a condition of progressive tuberculosis and is more extensively affected than that of Heifer 251.

II.

LESIONS OF THE LUNGS.

VIRUS.—B I.

Heifer 8.—As the result of an intravenous inoculation with an unknown but probably large dose of bacilli contained in an emulsion of tissues, the lungs are consolidated and show signs of breaking down, but do not exhibit the patchy distribution of caseating areas which is typical of the tubercular form of pneumonia. The lesions are obviously due to the action of the tubercle bacilli, which are present in enormous numbers.

Heifer 10.—In this case, another intravenous experiment, a typical miliary tuberculosis of the acute type and becoming confluent, has been produced. The material inoculated into this animal was a pure culture, not, as with Heifer 8, an emulsion of tissues. Heifer 10 may be compared with Heifer 12, which was inoculated subcutaneously with the same dose of the same culture. When Heifer12 was killed, 95 days after inoculation, no tubercles or tubercle bacilli could be detected in the lungs. This is therefore a striking instance of the greater severity of the intravenous method of inoculation. Another point worth recalling is that the same doses of the same culture were also inoculated into each of two quarters of the udder of Cow 40. Attention has already been drawn to the chronic character of the lesions found in this udder and the rarity of bacilli; it has also been noted that the culture used was 57 days old. Putting these three cases together, the inference seems justifiable that the bacilli were not in a vigour condition at the time of inoculation and that their vigour was quickly restored in the intravenous experiment but not in the other two.

Heifer 26.—The lesions are of the chronic type, consisting of isolated tubercles which are surrounded by a broad fibrous zone and contain few bacilli. The mode of inoculation was intravenous, the material injected being an emulsion of the udder of Cow 40.

Heifer 30.—This animal was inoculated subcutaneously with the same dose of the same material as Heifer 26. The lesions in the lungs are identical in histological characters with those found in Heifer 26. Both animals were killed 115 days after the commencement of the experiment.

Cow 40.—The lesions are of the chronic type, consisting of isolated, caseo-calcareous nodules, poor in bacilli, and surrounded by a dense fibrous zone. This condition of the lungs was produced 92 days after intramammary inoculation; the lesions in the udder, as noted above, are also of a chronic, fibro-calcareous type and contain very few bacilli. The resistance of the mammary tissue was not, however, sufficiently great to prevent dissemination.

Calf 48.—The strain with which this animal was inoculated had passed successively through Monkeys 2, 20, and 18. The lesions produced in the lungs are isolated, circumscribed, and not apparently progressive. The dose inoculated was not determined, therefore no definite opinion can be formed as to the influence of the passage through the monkeys.

Calf 52.—This animal was inoculated from Monkey 2, *i.e.*, two stages earlier in the monkey passage. The lesions in the lungs are decidedly more severe in type than those present in Calf 48.

Cow 74.—The lungs show very advanced progressive caseation and contain enormous numbers of bacilli. This condition of the lungs was found when the animal died, 299 days after intramammary inoculation. It has already been noted that the udder contains large numbers of bacilli, but shows a relatively small amount of tissue destruction. Heifers 26 and 30 and Cow 74 were all inoculated with the same material, viz., an emulsion of the udder of Cow 40. Heifers 26 and 30 each received 10 c.c., whilst Cow 74 received 10 c.c., into each of two quarters of the udder. Comparing the results, it is found that none

of the animals developed a rapidly fatal infection. Both the subcutaneous and the intravenous heifers showed a disseminated but distinctly chronic type of lung infection, when killed after 115 days; the cow died in 299 days with very advanced lung disease.

Calf 132.—The lungs show very acute infection. This is consistent with the high virulence of the virus, as established by the 50 mg. experiments. Calf 132 was inoculated from Monkey 62, which had been fed with milk of Cow 74. It is noteworthy that these bacilli were isolated from a monkey (*i.e.*, a representative of man) infected by drinking cow's milk and that they proved highly virulent to the bovine; this result may be compared with equally acute types of bovine lung disease produced by inoculation with bacilli of human origin.

VIRUS.—B II.

Heifer 14.—Progressive lesions of a fairly acute type have been produced.

VIRUS.—B III.

Cow 68.—The lungs show an early stage of progressive miliary tuberculosis. This condition was produced 48 days after intramammary inoculation.

Calf 210.—This animal was killed 143 days after subcutaneous inoculation with ·02 mg. of culture. The lung contains many small tubercles of a chronic, quiescent type, in which bacilli are still present. The mild character of the infection is obviously attributable to the smallness of the dose.*

VIRUS.—B IV.

Calf 140.—The lungs are practically free from tuberculosis. This is a good example of successful immunisation. The animal had been inoculated on six successive occasions, the last dose consisting of 229 mg. of virulent culture.

Cow 172.—There is only very slight tuberculosis of the lungs. The animal was killed 392 days after intramammary inoculation.

VIRUS.—B IX.

Calf 380.—Extensive tuberculosis, of a chronic type, has been produced in the lungs. The interesting feature of this case is that the lesions were produced by feeding with 1 mg. of culture.

VIRUS.—H 2. Sp. A.

The origin of this virus is exceptional, and must be considered before the histology of the lesions produced by it can be interpreted. It was, to begin with, a mixed virus and was obtained, during life, from various cases of human pulmonary tuberculosis. The material collected from them was sputum. It is therefore impossible to trace definitely the origin of all the various bacilli of which this mixture was composed. A large number of experiments have been performed with it. For the purpose of histological study, the lesions produced in any given case are considered with reference to the character of the bacilli exhibited in the animal which yielded the material inoculated.

Cow 63.—This animal was inoculated subcutaneously with about 160,000,000 bacilli derived from the tissues of Calf 89. Calf 89 was a case of very acute tuberculosis following intravenous inoculation. The lungs of Cow 63 do not show a very advanced or rapidly progressive type of tuberculosis; bacilli are scanty in number. There is, however, an abundant deposit of fibrin. This is generally a sign of acute infection, but the amount of tissue damage does not seem enough to account for the animal's death, 45 days after inoculation.

Calf 83.—This animal was inoculated subcutaneously with a dose of about 300,000 bacilli obtained from the glands of Calf 79. Calf 79 was killed when in a condition of moderately extensive generalised tuberculosis. The lungs of Calf 83 show outgrowths on the pleural surface and numerous lesions in the lung substance. The lesions are extensive and progressive in character and show a marked tendency to cell proliferation. Tubercle bacilli are abundant and fibrin is present. The effect of the inoculation, therefore, was severe, though the dose was small.

Calf 85.—This animal received a subperitoneal inoculation with the same dose of the same material as Calf 83. The lesions in the lungs are small, isolated, and retrogressive. This is a very different result from that obtained with Calf 83. With these small doses of virulent bacilli the effect produced seems to depend sometimes upon accidental circumstances; one may suppose that when the bacilli are able to commence multiplying without any initial delay they lead to severe infection, but when for one reason or another, their propagation is retarded to begin with, the animal is able to maintain a relatively more successful power of resistance.

Calf 89.—The inoculation in this case was intravenous, the same dose of the same material as in the two preceding cases being used. Acute tuberculosis has been produced. The effect was more severe than in the calf subcutaneously inoculated (Calf 83). Both animals were killed when moribund, but Calf 83 survived for 134 days and Calf 89 for only 23 days.

Calf 93.—This animal was inoculated intraperitoneally with the same material as the three preceding animals, but with double the dose. Advanced, progressive tuberculosis has been produced, with calcareous deposits in the older foci; bacilli are abundant, and there is a well marked deposit of fibrin. The bacilli, therefore, have again demonstrated their virulence, when tested by the intraperitoneal method of inoculation.

Calf 153.—This animal was inoculated subcutaneously with the tissues of guinea-pigs which had been inoculated with calcareous glands, very poor in bacilli, obtained from Heifer 13. The lungs of Calf 153 contain numerous discrete but progressive tubercles. The bacilli from the glands of Heifer 13 were therefore capable of producing generalised infection.

VIRUS.—H 7. C.M.

Calf 5.—This animal was killed 109 days after subcutaneous inoculation. The lungs show advanced, progressive tuberculosis, but do not contain many bacilli. This disease was produced by the inoculation of human tissue, *viz.*, an emulsion of human mesenteric glands.

Calf 101.—The lungs are in an early stage of acute infection. The animal was inoculated intravenously with a dose, estimated at 48,000 bacilli, of tissue emulsion from Calf 5.

Calf 103.—This animal was inoculated subcutaneously with the same dose of the same material as Calf 101. According to the post-mortem notes the lungs were affected, but not severely. In the specimens which I have microscoped there is no decisive evidence of tuberculosis.

Calf 105.—This animal also was inoculated subcutaneously with the same dose of the same material as the two preceding calves. The condition of the lungs resembles that found in Calf 101, but bacilli are less plentiful. This case, in its close resemblance to the result of an intravenous inoculation, shows very strikingly how readily tubercle bacilli find their seat of election in the lungs, even when the inoculation is subcutaneous and the dose very small. The difference between Calves 103 and 105 may be compared to the difference mentioned above (H 2. Sp. A.) between Calves 83 and 85. When a small dose of virulent bacilli are injected, the amount of damage they cause seems to be dependent, *ceteris paribus*, on whether or no there is an initial delay in their multiplication.

Calf 109.—This animal was inoculated intraperitoneally with the same dose of the same material as the three preceding animals. Acute miliary tuberculosis is present. The bacilli, therefore, have again proved their virulence when tested by the intra-peritoneal method.

* Numerically, the bacilli would amount to many millions, but culture bacilli are less potent than an equal number of tissue emulsion bacilli.

Calf 141.—Acute tuberculosis is present. The interest of this case lies in the fact that Calf 141 was inoculated from the supramammary gland of Cow 73. Cow 73 received an intra-mammary inoculation, but no tuberculosis of the udder was found post mortem. The potential virulence of the material inoculated is established by the experiment on Calf 141.

VIRUS.—H 8. S.C.

Calf 305.—The virus inoculated has been shown by the 50 mg. culture inoculations to be of low virulence. Calf 305 was inoculated intravenously with a large dose (about 730,000,000) of bacilli contained in a tissue emulsion, and was killed, when moribund, 26 days afterwards. The lungs are consolidated and contain large numbers of tubercle bacilli, but have not the typical appearance of the tubercular form of pneumonia.

Calf 361.—This animal was killed 76 days after intravenous inoculation with a tissue emulsion containing 41,500,000 bacilli. The lungs are crowded with typical miliary tubercles of a chronic type. This is a good instance of histologically typical tuberculosis, not of an acute type, following intravenous inoculation with a virus of relatively low virulence.

VIRUS.—H 9. C.T.

Calf 185.—This is another virus which the 50 mg. culture inoculations have shown to be of low virulence. Calf 185 was inoculated subcutaneously with a large dose of an old broth culture, and was killed 40 days afterwards. The lung contains small, slightly caseous tubercles in which bacilli are scanty. This is one of many instances in which a mild type of disseminated tuberculosis has been produced by the inoculation of feebly virulent material.

VIRUS.—H 10. B.S.

Heifer 81.—This animal was inoculated subcutaneously with an emulsion of human mesenteric glands (dose not estimated) and was killed 132 days afterwards. The lungs contain tubercles of a chronic type which are similar to those produced in Heifers 26 and 30 after inoculation with a bovine virus.

Calf 191.—Advanced, progressive, typically tubercular pneumonia is present. This result is consistent with the high virulence of this virus as established by the 50 mg. inoculations.

VIRUS.—H 12. H.N.

Calf 319.—This is another virus which is shown by the 50 mg. inoculation tests to be of relatively low virulence. Calf 319 was inoculated intravenously with a tissue emulsion containing over 100,000,000 bacilli and was killed, when moribund, 57 days afterwards. The lungs are in a condition of miliary tuberculosis advancing to general consolidation. Enormous numbers of tubercle bacilli are present, but no fibrin has been demonstrated, and the amount of tissue destruction is small relatively to the large number of bacilli present. This lung may be compared with the lung of Calf 305 (H 8, S.C.). The following points are of interest. Calf 305 only survived for 26 days, *i.e.*, it can hardly be said to have survived the initial severity of the experiment; Calf 319, however, remained alive for 57 days and allowed a further development of the disease. This further development, though not marked by great tissue destruction, is more in the direction of a typical tuberculosis.

VIRUS.—H 13. A.D.

Calf 301.—The lungs show an acute, rapidly progressive tuberculosis, associated with fibrin formation and the presence of large numbers of bacilli. This case is of interest. Calf 301 was inoculated subcutaneously with a large dose (over 540,000,000) of bacilli contained in the organs of guinea-pigs, which were the third series of guinea-pigs inoculated from Calf 129. Calf 129, inoculated with a very small dose (16,000 bacilli) of original material, showed very slight disease. Calf 119, the control animal to Calf 129, showed about the same amount of disease, and Calf 225, inoculated with 3,500,000 bacilli obtained from Calf 119, showed no disseminated lesions. The earlier experiments, therefore, made with the doses mentioned, showed very little virulence for the bovine. But Calf 301, inoculated with a very much larger dose, showed very severe infection. Calf 299, inoculated intraperitoneally with the same dose of the same material as Calf 301, showed much less dissemination of the disease than Calf 301. The same material was also inoculated intraperitoneally, in a dose of 135,000,000 bacilli, into Rabbit 28; this animal died of general tuberculosis in 24 days. Taking these three cases together, the material inoculated proved to be of high pathogenicity in two cases (Calf 301 and Rabbit 28), but of lower pathogenicity in a third. On the question whether this absence of complete uniformity in results was accidental or characteristic of some instability in the material inoculated, some light may be thrown by considering the effects of inoculations with cultures isolated from Calf 301. Injected in doses of 50 mg., these cultures failed to kill bovines in 90 days; the animals were, clinically, "well" at the end of that period. Injected in doses of 10 and 1 mg. into the peritoneal cavity of the rabbits, they did not produce death in 90 days. This evidence, then, so far as it goes, suggests that the virulence of the bacilli present in Calf 301 was not a stable characteristic.

Calf 325.—This animal was inoculated subcutaneously with an emulsion from the tissues of Calf 301 containing about 500,000,000 bacilli and was killed, when moribund, 24 days afterwards. The lungs show highly acute tuberculosis. This result, therefore, corroborates the virulent capacity of the bacilli as exhibited in Calf 301. Moreover, a smaller dose ($3\frac{1}{2}$ million) of the bacilli from Calf 301 produced fatal disease in another Calf (321) in 63 days. The pathogenic power of the virus, therefore, appears to have acquired stability in its passage from Calf 301 to Calf 325 and Calf 321. Moreover, cultures, isolated from Calf 321 proved to be of high virulence for bovines.

VIRUS.—H 14. F.S.

Calves 121 *and* 125.—These animals both exhibit acute tuberculosis of the lungs as the result of subcutaneous inoculation with an emulsion of human tubercular mesenteric glands containing very large numbers of bacilli. The high virulence of this virus has been confirmed by the experiments with 50 mg. of culture.

Heifer 197.—Severe tuberculosis has been produced, after inoculation with about 85,000,000 bacilli contained in the tissues of Calf 121.

Heifer 243.—This animal was inoculated with 4,500,000 bacilli from Heifer 197. Although the dose was much smaller than with Heifer 197, severe, progressive disease has been produced.

VIRUS.—H 16. J.H.

Heifer 245.—The lungs show chronic, circumscribed tubercles. The dose inoculated was large (about 157,000,000 bacilli), and in the companion animal, Calf 423 A, produced very severe disease. Apart from the greater size of the heifer, the condition of pregnancy may partly account for the milder nature of the infection.

Calf 273.—The animal was killed 89 days after subcutaneous inoculation with 11,000,000 bacilli. The sections of the lung show caseous tuberculosis of the chronic, slowly progressive type, containing few bacilli.

Calf 281.—Calf 281 was inoculated subcutaneously with 11,000,000 bacilli from Calf 355, and was killed, when ill, 54 days afterwards. I have described the lesions in the lungs as being numerous, progressive, and moderately acute in type. Tubercle bacilli are moderately numerous. The infection is more severe than in Calf 273.

Calf 355.—This animal was inoculated subcutaneously with about 3,000,000 bacilli from Calf 273, and was killed, when very ill, 43 days afterwards. The lungs show disseminated tubercles of a progressive but not very acute type, and do not contain numerous bacilli. The infection is somewhat more severe than that found in Calf 273.

VIRUS.—H 17. Sp. B.

This virus, like H 2, Sp. A., was originally a mixture of viruses, and consisted of bacilli contained in the sputum of various hospital patients. As the starting point for the consideration of my histological results I take the virus as represented in Calf 339. After subcutaneous inoculation with a large dose, 166,000,000 bacilli, this animal developed only slight disease. This result, corroborated by a large number of earlier experiments, has established the fact that the bacilli present in Calf 339 were characterised by a low virulence for the bovine.

Calf 529.—This animal was inoculated intravenously with about 1,000,000,000 bacilli derived from Calf 339 by passage first through guinea-pigs, then through Calf 475, then through Calf 539. Calf 529 died 13 days after inoculation. The lungs are consolidated, and contain enormous numbers of tubercle bacilli. The bacilli have clearly multiplied very rapidly since their introduction into the body. As the experiment terminated at an early stage after a severe injection, it is impossible to place any useful interpretation upon the histological condition of the lungs.

Calf 555.—This animal was inoculated subcutaneously with about 150,000,000 bacilli derived from Calf 529 after subsequent passage through Calf 553. Calf 555 was killed, when moribund, 58 days after inoculation. The lungs show tuberculosis of a highly severe type, and are histologically identical with the lungs of animals which have been inoculated with 50 mg. of a highly virulent culture. Comparing Calf 555 with Calf 339, we find that the virus has been raised to a high degree of virulence by intravenous passage through the bovine.

Calf 607.—This was a bull-calf, nine months old, which was inoculated intravenously with 90 mg. of culture isolated from Calf 339, and was killed in good health 48 days afterwards. The lung alveoli show some irregular thickening of their walls, but no tubercles; very few bacilli are to be found. This result shows once more that the bacilli in Calf 339 were of low virulence for the bovine. This fact had already been fully established, but not by the application of such an extremely severe test as the intravenous inoculation of 90 mg. of culture.

Calf 685.—This, a younger animal than the last mentioned, and therefore presumably more susceptible to the effects of intravenous inoculation, was also inoculated intravenously with a culture of Calf 339. It was killed, in good health, 64 days afterwards. Again we find that only minimal lesions were produced. I describe the lesions in the lungs as consisting of a few small tubercles of a retrogessive character. Taking the last two cases in conjunction with all the earlier cases, the proof that the bacilli in Calf 339 were of low virulence to the bovine may fairly be described as overwhelming. It serves to place beyond all possibility of doubt the important fact that in the passage experiment from Calf 339 to Calf 555 there was a genuine and very marked increase of virulence.

VIRUS.—H 18. T.T.

Calf 165.—Many small tubercles, exhibiting retrogressive characters, are present. Like a great many other human viruses of relatively low virulence, this virus is capable of producing in the bovine disseminated tuberculosis of a mild type. Calf 165 was inoculated subcutaneously with an emulsion of human mesenteric glands containing about 4,500,000 bacilli.

Calf 405.—This animal was inoculated with a much larger dose (270,000,000 bacilli) than the last. The condition of the lungs indicates that there has been an invasion of tubercle bacilli which has been to a large extent overcome, and is being followed by a process of diffuse fibroid change. The result of this experiment therefore supports the indication afforded by Calf 165 that the virulence of this virus is not high.

VIRUS.—H 19. S.W.

Calf 159.—Extensive and progressive tuberculosis is present. This is in agreement with the virulence of the virus as exhibited by the 50 mg. inoculations.

VIRUS.—H 22. F.W.

Calf 399.—This animal was inoculated intravenously with a tissue emulsion containing over 700,000,000 bacilli, and was killed, when very ill, 34 days afterwards. The lungs exhibit a consolidation which is not typical of tubercular pneumonia, but is evidently due to the presence of enormous numbers of tubercle bacilli. No fibrin is present; it is noteworthy that when pneumonia is caused either by highly virulent tubercle bacilli or by pathogenic organisms other than the tubercle bacillus a fibrinous exudate is generally formed. Considered *per se*, the lesions present in this animal's lungs do not readily lend themselves to a definite interpretation. Some helpful information is obtained from the two succeeding experiments. Calf 457 was inoculated intravenously with 20,000,000 bacilli from the tissues of Calf 399; and Calf 505 was inoculated intravenously with 11,000,000 bacilli from the tissues of Calf 457. Calf 457 died in 57 days with "miliary tuberculosis of the lungs;" and Calf 505 was killed in 62 days with "three minute tubercles in the lungs." Calf 505 shows that 11,000,000 bacilli was not a large enough dose to be efficient, and that the extraneous material injected together with the bacilli caused no appreciable injury. Calf 457 shows that a large dose, 20,000,000, produced miliary tuberculosis. It seems, therefore, probable that the still severer affection produced in Calf 399 is attributable to the specific action of the much larger number of bacilli inoculated.

Calf 749.—This animal was inoculated intravenously with 46 mg. of culture and was killed, when very ill, 24 days afterwards. The lung is in a similar condition to that of Calf 399, but bacilli are less numerous. This last point is noteworthy, considering that the number of bacilli inoculated was enormously greater than in Calf 399. The further history of the bacilli in Calf 749 is of interest. 250,000,000 of them were inoculated intravenously into Calf 811, which was killed 49 days afterwards. The post-mortem examination revealed "No tubercles in lungs. Some caseous foci in thoracic glands." The bacilli were again carried on, intravenously, into Calf 817, with a dose of 890,000,000. The animal, killed after 31 days, showed "Lungs extensively mottled with irregular foci. Few tubercle bacilli in these and none in other organs." Comparison of these three experiments with the three preceding experiments brings out an interesting point. In the preceding set the bacilli were fewer in number but were, in proportion to their numbers, more effective; this series started with a tissue emulsion, whilst the later series started with a culture. This is an instance, then, of a virus, originally of low virulence, becoming still less virulent after residence on culture media. The significance of this fact is obvious in relation to modification experiments such as that commencing with Calf 339 (H 17. Sp. B.), which has been dealt with above. It has been shown to be possible to begin with a strain of low virulence and to greatly increase its virulence; but there is evidence against the assumption that if we return to a culture of this same strain we ought to be able to repeat the process.

VIRUS.—H 23. J.P.

Calf 365.—The lesions are chronic rather than acute in type and may probably be regarded as retrogressive. This is another instance of a mild type of disseminated tuberculosis produced by a virus which is only slightly virulent in doses of 50 mg. of culture.

Calf 441.—Numerous, small, retrogressive lesions are present, but contain very few bacilli. This result supports the opinion formed as to the nature of the morbid process in Calf 365.

VIRUS.—H 25. A.T.

Calf 551.—This animal (a bull-calf 10 months old) died 21 days after intravenous injection with 100 mg. of a virus which has been found in the 50 mg. subcutaneous inoculation experiments to be of low virulence. The lung is very congested and contains a fibrinous deposit; bacilli are plentiful but there are no tubercles. The relatively low virulence of the bacilli is shown by the absence of any definitely tubercular lesions. Owing to the early termination of the experiment it is impossible to decide whether the bacilli were capable of giving rise to genuine tubercles in this animal.

Calf 831.—This animal died 17 days after intravenous inoculation with 1,000 000 bacilli from Calf 551. The lungs are congested and contain large numbers of bacilli but no tubercles. Death can hardly be attributable to the specific action of the bacilli.

Calf 859.—This animal died 19 days after intravenous inoculation with 2,000,000 bacilli from Calf 831. The lungs are consolidated, and this condition appears due to the presence of enormous numbers of tubercle bacilli, but the lesions are not typical of tubercular pneumonia. No fibrin is demonstrable. Taking this and the two preceding cases together, the main result appears to be negative. The early production of fatal results cannot be regarded as representative of the specific action of the bacilli; on the other hand, it affords no evidence that these bacilli might not, under more favourable circumstances, have given rise to a typical tuberculosis.

VIRUS.—H 29. M.F.

Calf 477.—Acute progressive tuberculosis has been produced after subcutaneous inoculation with a large dose of tissue emulsion bacilli. This result is in conformity with the high virulence of the virus when inoculated in doses of 50 mg. of culture.

III.

LESIONS OF THE LIVER, KIDNEYS AND LYMPHATIC GLANDS.

These organs have been examined in detail with the object of gaining further information about the extent of dissemination of the disease and the characters of the lesions produced. It has generally been found that they confirm, on the whole, the information afforded by an examination of the lungs. When the lungs exhibit acute disease it is usual to find the same type of infection in the liver and lymphatic glands, whilst the kidneys, though generally tubercular, frequently do not exhibit lesions of equal severity. When the lungs exhibit disease of a less severe type it is usual to find corresponding indications of less severity in the other organs examined. The majority of the specimens examined illustrate these general principles and do not call for individual comment. Attention is called below to specimens which present some features of special interest.

VIRUS.—B I.

Cow 40.—The supramammary and iliac glands exhibit like the udder, into which the inoculation was made, the caseo-calcareous type of lesions, with numerous giant cells. It is noticeable that bacilli are much more numerous in the lymphatic glands than in the udder.

Cow 74.—In the supramammary gland, as in the udder, where the inoculation was made, the lesions are peculiar in that, though progressive, they exhibit a relatively small amount of tissue destruction in proportion to the large number of bacilli present.

Calves 146, 154, *and* 156.—These calves were fed with bacilli contained in milk, each receiving a dose of from 5,000,000 to 10,000,000. They were all killed 36 days afterwards. The nature of the lesions in the mesenteric glands does not appear to me to justify the assumption that the disease is quiescent or retrogressive. It seems possible that if the animals had been allowed to live longer a wider dissemination might have occurred than was found post-mortem.

VIRUS.—B II.

Calf 50.—This animal was killed 151 days after the commencement of a feeding experiment (sucking cows with infected udders.) The mesenteric glands show caseo-calcareous lesions of the chronic type; they still contain bacilli and are not completely avascular. The post-mortem examination showed that "pea-sized tubercles were present in the lungs (about two dozen), numerous in the liver, and scanty (about eight to twelve) in the spleen."

Cow 500.—This animal was killed, when very ill, 32 days after intramammary inoculation. An external iliac gland contains early lesions of an acute type, containing bacilli in association with recent deposits of fibrin.

VIRUS.—B III.

Calf 116.—This animal was killed 92 days after the commencement of a feeding experiment (sucking Cow 68). The mesenteric glands show lesions of a chronic type, but contain relatively large numbers of bacilli. The post-mortem notes state, "One tubercle was seen on right posterior lobe of lung; one also in spleen. Other organs and glands normal."

VIRUS.—B IV.

Calf 140.—The almost complete absence of tubercular lesions in the specimens of liver, kidney, and various lymphatic glands which I have examined confirms the successful establishment of immunity in this animal.

Calf 188.—This animal was fed with a small dose (1,000,000) of bacilli contained in milk, and was killed forty-four days afterwards. The mesenteric gland which I have examined shows tuberculosis of the chronic type with marked evidence of retrogressive change. At the post-mortem examination all the organs and glands were found to be normal with the exception of three mesenteric glands and one colic gland.

VIRUS.—B IX.

Calf 380. The liver, kidney, and left bronchial gland contain caseous tubercles, of a chronic type, in which bacilli are rare. Similar lesions are present in the lungs. The feature of special interest about the case is that this disseminated disease was produced by feeding with 1 mg. of culture.

VIRUS.—H 1. C.M.

Cow 3.—Small, retrogressive tubercles, containing a few bacilli have been found in a supramammary gland. In this animal extensive tuberculosis of the udder was produced after intramammary inoculation. The disease has travelled to the nearest glands, and there seems to have been arrested.

Calf 21.—Calf 21 was allowed to suck Cow 3 for sixty-two days and was killed 226 days after the commencement of the experiment. Specimens of the mesenteric glands show isolated, retrogressive tubercles, in which bacilli are very rare. At the post-mortem examination no generalisation of the disease was found.

VIRUS.—H 2. Sp. A.

Heifer 11.—This animal was fed for seven months with mixed human sputum and was killed 209 days after the commencement of the experiment. The mesenteric glands show progressive tuberculosis of the chronic, caseo-calcareous type. At the post-mortem examination lesions were found in the hepatic glands and on the surface of the lung.

VIRUS.—H 7. C.M.

Cow 73.—The supramammary gland shows caseo-calcareous tubercles surrounded by a broad fibrous zone. Bacilli are present but not numerous. This animal had received an intramammary inoculation, but at the post-mortem examination the udder showed no definite evidence of tuberculosis.

VIRUS.—H 8. S.C.

Calf 267.—This animal was inoculated subcutaneously on the right side of the neck with a culture killed by heating. The animal was killed 111 days afterwards. I have not been able to find either tubercles or tubercle bacilli in the right prescapular gland. This negative result was obtained with a virus known to be of low virulence for the bovine. The absence of any lesions in the nearest gland after the inoculation of dead bacilli emphasises the fact that the pathogenicity of these bacilli in the living condition, though low, is real and specific.

Calf 305.—This animal was killed, when very ill, twenty-six days after a large intravenous inoculation. The liver shows early tubercles which exhibit a fibrinous deposit. The amount of tissue destruction is small relatively to the large number of bacilli present. There is an interstitial infiltration of tubercle bacilli in the kidney, which may be compared with the lesions produced in the kidneys of Calf 132 (B 1) and Calf 101 (H 7. C.M.). Bacilli are numerous in lymphatic glands selected from various parts of the body and the bronchial and posterior mediastinal glands show acute infection. After making due allowance for the influence of lowered resistance in a young animal inoculated with a large dose intravenously, these results are not without weight in demonstrating the pathogenicity for the bovine of human tubercle bacilli possessing a relatively low virulence.

Calf 361.—This animal, killed 76 days after intravenous inoculation, shows typical tubercles of a chronic type in the liver, kidneys, and lymphatic glands.

VIRUS.—H 12. H.N.

Calf 319.—This animal was killed, when moribund, fifty-seven days after intravenous inoculation with something over 100,000,000 bacilli. The liver and kidney show very little tissue reaction and no fibrin, but are swarming with bacilli. Two features are noteworthy:—(1) The feeble attacking powers of the bacilli, and (2) the inability of the animal to arrest their multiplication, even after fifty-seven days, a period long enough to allow the calf to recover from the initial disturbance due to the severity of the injection. In this experiment, therefore, human bacilli of low virulence proved their capacity for surviving and actively multiplying within the bovine organism.

VIRUS.—H 17. Sp. B.

Calf 529.—This animal died thirteen days after intravenous injection with 1,000,000,000 bacilli. The liver shows diffuse necrosis suggestive of a toxic infection. In both liver, kidneys, and lymphatic glands tubercle bacilli are present. No definite interpretation can be placed on the action of the bacilli, owing to the early termination of the experiment.

VIRUS.—H 22. F.W.

Calves 399 *and* 749.—Both these animals received intravenous injections with large doses. The kidneys, liver, and lymphatic glands show only a small amount of tissue damage, though bacilli are plentiful. Both the amount of tissue damage and the numbers of bacilli appear greater in Calf 399 than in Calf 749. Calf 399 received 700,000,000 tissue emulsion bacilli and was killed thirty-four days afterwards, whereas Calf 749 received a much larger dose of culture (46 mg.) and was killed twenty-four days afterwards.

VIRUS.—H 25. A.T.

Calf 551.—This animal (ten months old) died twenty-one days after intravenous inoculation of 100 mg. of culture. The liver contains moderately numerous bacilli (not dense aggregations) and shows many small foci of slight tissue destruction which have not advanced to the formation of definite tubercles. In the kidney a few bacilli are present but they have produced no evidence of tissue damage. In a thoracic gland bacilli are present in small numbers, but have produced hardly any tissue change. The bacilli contained in this enormous dose of culture, therefore, have not only produced very little tissue destruction but show little or no evidence of active multiplication.

Calf 831.—This animal died seventeen days after intravenous inoculation with about 1,000,000,000 bacilli from the tissues of Calf 551. The liver, kidney, and lymphatic glands show no noteworthy tissue damage but contain more bacilli than the corresponding tissues of Calf 551, notwithstanding the, numerically, very much smaller dose of bacilli.

Calf 859.—This animal died nineteen days after intravenous inoculation with about 2,000,000,000 bacilli from tissues of Calf 831. The liver and kidney show only a slight amount of tissue damage and contain much fewer bacilli than the corresponding organs of Calf 831.

CONCLUSIONS AND COMPARISONS.

I call attention to the following results which arise out of my histological examination of lesions produced in the bovine under different experimental conditions.

The Underlying Unity of the Morbid Process.

The lesions which I have examined, though presenting much diversity in character, are typical of tuberculosis.* They exhibit the features which are admitted by all histologists, whatever their theories as to the histogenesis of the tubercle, to be characteristic of the morbid process known as tuberculosis. There is nothing new or surprising about this fact; it is in harmony with the general consensus of scientific opinion on the histology of this disease. But it is a fact which deserves emphasis, because it has an important bearing on the experimental study of bovine and human viruses. No differentiation of viruses derived from these two sources can be regarded as valid

* This statement is not intended to include some of the rapidly fatal results produced by the intravenous inoculation of young calves with large doses.

if it fails to take into account the histological unity of the morbid processes which they induce.

The Characteristics of the Bovine Disease.

Under natural conditions bovine tuberculosis is not usually a rapidly fatal disease. In the great majority of cases it runs a very long course; very frequently the disturbance to the general health is no more than slight, and the lesions set up become quiescent or definitely retrogress. This feature of bovine tuberculosis is illustrated by numerous cases of the experimentally produced disease which I have examined. In many instances I have shown that the lesions produced, sometimes after inoculation with a bovine virus and sometimes after inoculation with a human virus, show marked evidence of conservative change; some of these lesions appear to be slowly progressing, but are obviously meeting with a vigorous tissue resistance; others, again, may be described as quiescent; and in many instances the lesions can be definitely pronounced to be retrogressive.

In the second place, the spontaneous disease may assume a highly acute type. To this condition numerous experimental parallels have been obtained, and I have described in detail the histological characters of the lesions produced. The most interesting of these cases are those where the dose inoculated has been kept within moderate limits. These cases show that acute infection may sometimes be set up by introducing into the tissues a number of bacilli which is relatively small, or at least is not enormously greater than the number which is likely to be introduced into the bovine organism by any of the channels of natural infection.

In the third place, infection has been set up in young calves by subcutaneous inoculation with enormous doses of bacilli. Under these special experimental conditions the morbid process tends to follow one of two courses. (1) Either the local resistance at the site of inoculation is rapidly overcome, and acute disseminated tuberculosis ensues, or (2) the local resistance is successful in preventing the further invasion of the great mass of the inoculated material, and the relatively few bacilli which become disseminated only succeed in setting up small, chronic, and often definitely retrogressive lesions.

The Conditions determining the Severity of Infection.

In comparing my histological results with the experimental conditions under which the lesions I have examined were produced, I find that there are several factors which have helped to determine the degree of severity of the morbid process induced. Taking the subcutaneous inoculations as being the most important, the chief of these conditions are—(1) the natural virulence of the virus; the differences in the virulence of different viruses have proved to be extremely great, and this is obviously the most important factor: (2) the dose; *ceteris paribus* a large dose is more likely to produce severe disease than a small dose, but a small dose of a virulent virus may produce severe disease, whereas, with a virus of low virulence, mere increase of the number of bacilli inoculated subcutaneously has not been found to produce disseminated lesions of an acute type: (3) the age of the animal; it has conclusively been shown that young calves are much more susceptible than older animals: (4) accidental or unknown circumstances, such as "individual susceptibility." To these may be added (5) the suggestion, based on theory rather than on actual proof, that the condition of vitality of the bacilli inoculated is occasionally a factor of importance; bacilli which are in a condition of active multiplication when inoculated are more likely to continue to grow quickly than those which are not in this condition, and the more rapidly the bacillus commences its growth the more likely is it to overcome the bovine resistance.*

Progressive and Retrogressive Lesions.

Tuberculosis as a disease is especially characterised by the great variety in the degrees of severity which the morbid process may assume. At the one extreme we have lesions characterised by rapidly progressive necrosis and the presence of large numbers of bacilli; and at the other extreme we may have lesions which are shown by guinea-pig inoculations to contain no living bacilli; histologically these lesions may be so far "repaired" as to be no longer definitely recognisable as tubercles, or they may be characterised by a dense fibrous barrier which completely circumscribes a focus of caseous or calcareous material. Between these extremes there are conditions where progress is obviously taking place, but more or less slowly, conditions where it is impossible to say whether the lesions have attained their maximum extension or not, and conditions where the conservative changes appear, more or less definitely, to be gaining the upper hand. In other words, just as, clinically, it is often impossible to say definitely whether a case is "cured" or not, so, histologically, it is often impossible to give a categorical "Yes" or "No" in reply to the questions—Has the animal overcome the bacillus or is the bacillus going to overcome the animal? In summarising my histological results I have endeavoured, as far as possible, to give unambiguous answers to these questions; but one ought not to forget the common liability of tuberculosis to "recrudesce" from a focus which is apparently quiescent; and in association with this consideration I call attention to the significant fact that many of these bovine tubercles of the "chronic" or "retrogressive" type not only contain bacilli but are still well vascularised.

Bovine Lesions produced by Human and by Bovine Viruses.

Amongst the animals inoculated with human viruses I have found every variety of lesion, ranging from the highly acute to the obviously retrogressive; and amongst the lesions produced by bovine viruses I have found exact counterparts for every one of these. If, therefore, we may assume that causes which produce identical effects are identical in character, these investigations provide strong evidence that the bovine bacillus is capable of infecting man.

Comparison of Bovine Resistance with Human Resistance.

Tuberculosis is very common amongst both bovines and man, but these investigations indicate that the bovine powers of resistance are higher than those of man. All the viruses investigated of bovine origin have been found, when tested under conditions which favour the development of their maximum virulence, to be very highly pathogenic; whereas, of human viruses, tested under equally favourable conditions, some have proved to be of high and others of low virulence. It seems, therefore, that under the conditions of natural infection, bacilli cannot usually obtain a permanent residence in the bovine unless they are of high virulence; whereas under the conditions of natural infection which prevail for man, bacilli of much lower virulence can also permanently establish themselves and can eventually produce fatal disease.

Increase of Virulence due to Residence in Bovine Tissues.

In the case of three human viruses I have reported on specimens which appear to indicate that their virulence has been increased by prolonged residence in the tissues of the bovine.

Susceptibility, Tolerance, and Resistance.

When an animal is highly susceptible to the bacilli inoculated, they multiply freely, and when it is highly resistant they are, as a rule, only found in scanty numbers. There is thus a general relationship, in most cases, between the virulence for a particular animal of the bacilli inoculated and their capacity for multiplication within its tissues. But in certain cases I have found that the bacilli have been able to grow freely, but have produced a relatively small amount of tissue destruction, and the animals have survived the infection for a long period. It is noteworthy that in these cases a very large number of the bacilli are intra-cellular. This condition may be regarded as illustrating the tolerance of the bovine organism for the bacillus and may be compared with the same phenomenon which is met with much more commonly in the rat. Cow 74, which was inoculated with a bovine virus, exhibits this tolerance in an interesting manner, and I have also found that in many cases of

* The relatively mild type of disease set up by inoculation of Cow 40 with an old culture of B 1 (a virulent virus) may perhaps be taken as an instance in point.

intravenous inoculation with human viruses of low virulence there is again a very abundant increase of bacilli associated with a relatively small amount of tissue destruction.

Intravenous Inoculations with Human Viruses.

I have examined several specimens of lesions produced in animals, usually calves a few weeks old, which have been inoculated intravenously with large doses of bacilli known to be of low virulence when inoculated subcutaneously. In some of these cases bacilli are swarming all over the body and the animal has died in a few weeks, though no lesions have been produced which can be regarded as typical of tuberculosis. In other cases, where the animal has lived longer, bacilli are again very numerous and lesions have been produced which are more or less characteristic of tuberculosis, though the amount of tissue destruction is usually small relatively to the number of bacilli present. In other cases, again, the animal has successfully resisted the infection, bacilli are scanty, and there is no tubercle formation. And occasionally, as in Calf 361 (H. 8 S.C.) typical tuberculosis, histologically of a chronic type, has been produced in all the organs of the body. With regard to the histological interpretation of these results I do not feel prepared to offer a definite opinion. The experimental material provided is not sufficient to determine satisfactorily the susceptibility of the bovine to viruses inoculated by this method. All that can be said about the results obtained is that in some cases death took place too soon to afford any useful indication of the morbid processes at work, in other cases tuberculosis was set up, and in others, again, the animals exhibited a high degree of resistance.

EXPERIMENTS ON ANTHROPOID APES AND MONKEYS.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Fed.—Baboon 2.

Baboon 2 (young) was fed with 1,000,000 bacilli contained in the milk of Cow 64 and was killed 96 days afterwards.

Lungs.

Histological Changes.—The section examined passes through a tubercle, about 3 mm. in diameter, which is situated close to a bronchus and is caseous and necrotic except at its periphery. There is some fibrous tissue surrounding it, but external to this tissue are small, irregular areas of consolidation which are commencing to caseate. Both these areas and the outer zone of the tubercle contain many giant cells. The adjacent lung tissue is partially collapsed. The general appearances indicate that the caseous process has not been arrested, but is extending from the original focus. Multinuclear leucocytes and their remains are very abundant within the lesions. Plasma cells are rare and stain badly.

Distribution and Characters of Bacilli.—Bacilli are numerous, especially in the peripheral part of the tubercle and within giant cells. They are from 2 to 3μ in length; some of them are beaded, and many of the longer forms are curved.

Liver.

Histological Changes.—Miliary tubercles are present. They are small, infrequent, and just commencing to caseate. They only contain a small number of leucocytes.

Distribution of Bacilli.—Bacilli are present in these tubercles, but are very rare.

Kidneys.

No lesions found.

Mesentery.

Histological Changes.—The mesentery is very greatly thickened and consists of fibro-caseous material infiltrated with leucocytes and lymphocytes. The leucocytes occur in large masses and, except where disintegrated, show a well-marked oxyphil granulation. Most of them are multinuclear, but the form with a spherical, deeply stained nucleus also occurs.

Distribution of Bacilli.—Bacilli are present, but rare.

Mesenteric Gland.

The gland examined is only slightly affected, containing a few small patches of commencing necrosis in which a few bacilli have been found.

SUMMARY

Disseminated tuberculosis, not very acute in type, but apparently progressive.

VIRUS.—B I.

Animal Fed.—Baboon 6.

Baboon 6 (young) was fed with 10,000,000 bacilli contained in the milk of Cow 64, and was killed 67 days afterwards.

Lungs.

The piece of tissue examined shows thickening and infiltration of the alveolar walls with lymphocytes and leucocytes, but no tubercles and no tubercle bacilli.

Liver.

In the tissue examined one minute, doubtful lesion is present, but contains no bacilli.

Kidneys.

No lesions or bacilli found.

Mesenteric Glands.

Histological Changes.—The gland examined shows large caseous nodules. At the periphery of these, giant cells are very numerous. There is a dense infiltration of multinuclear leucocytes.

Distribution of Bacilli.—Extremely rare.

SUMMARY.

There is some evidence of dissemination, but the disease appears to be retrogressive.

VIRUS.—B I.

Animal Fed.—Baboon 8.

Baboon 8 (adult) was fed with 1,000,000 bacilli contained in the milk of Cow 64, and was killed 97 days afterwards.

Lungs.

The lungs are packed with tubercles. The largest of them are several millimetres in diameter, circular in outline, and almost completely caseous. Smaller tubercles of various sizes are also numerous, the smallest being about the size of a distended alveolus. These smaller tubercles are often grouped together, forming patches of almost complete consolidation, and are exceptionally rich in large giant cells. In the rest of the lung tissue there is considerable desquamation of epithelial cells, and many of the bronchioles are filled with cellular débris.

In most of the tubercles bacilli are found, but only in small numbers. Their most frequent situation is within giant cells. In the large caseous tubercles bacilli occur more frequently at or near the margins than amongst the caseous material. In no parts of the tissue do the bacilli occur in groups. There is, therefore, no evidence that the bacilli are undergoing rapid multiplication.

Multinuclear leucocytes are abundant everywhere, in the alveolar walls, within the lumen of the alveoli and bronchioles, and in the tubercular lesions. In the older tubercles there is a dense zone of these cells bordering on the area of complete necrosis. In the earlier lesions they do not form a definite zone, but are scattered about everywhere. The protoplasm of these cells is oxyphil, and some of them show a well-marked granulation. There is also a copious infiltration of small lymphocytes. These cells are most abundant in areas which have not commenced to break down. Plasma cells form a definite outer zone to the older tubercles, are very abundant in the neighbourhood of the outer walls of the bronchioles, and are distributed plentifully but irregularly amongst the smaller tubercles. Very little increase of adult fibrous tissue is demonstrable in van Gieson specimens. In many of the occluded alveoli the epithelial cells have reverted to the embryonic type, and cells of this type are plentiful in the earlier tubercles. The earlier tubercles are also permeated with cells, possessing fusiform or oval nuclei, which are probably derived from the capillary endothelium.

The giant cells exhibit a good deal of diversity in the

types and distribution of their nuclei. In many places a giant cell, with large, more or less spherical nuclei, is found filling up the lumen of an alveolus, the wall of which can still be recognised by the persistence of its blood capillaries ; in these cases the giant cell appears undoubtedly to arise from a fusion of alveolar epithelial cells, accompanied by a multiplication of their nuclei. In other cases this relationship to an alveolus cannot be made out, and the nuclei of the giant cell are of a smaller and more elongated type, strongly suggestive of an endothelial origin. And in many other of the giant cells more than one type of nucleus is found. The nuclei may be either heaped up in the centre, formed into a circumferential ring, or crowded into one end of the cell. This diversity of appearance suggests the view that the giant cell is a degeneration product formed from a fusion of cells which may be of more than one type and are, at all events in some cases, epithelial and in some others endothelial.

Liver.

There are a few small tubercles, the majority of them interlobular. The larger of them are caseous, and they nearly all contain a few giant cells.

Tubercle bacilli are very rare ; a few have been found within giant cells.

The tubercles exhibit only a slight degree of infiltration with oxyphil leucocytes and lymphocytes. Some of the giant cells appear from the character of their nuclei to be of endothelial origin ; whilst others are undoubtedly formed from parenchymatous cells. There is no definite plasma cell reaction.

Kidneys.

There is a general slight increase of interstitial tissue ; and in the cortex there are several small patches of denser infiltration, some of which have involved the renal tubes.

No tubercle bacilli have been found.

The lesions are not histologically tubercular.

Lymphatic Glands.

Submaxillary.—The greater part of the gland is occupied by extensive and partially confluent areas of advanced caseation. There is no fibrous tissue demarcation of these areas, but a great many giant cells are found near to their periphery.

Most of the giant cells contain a few bacilli ; bacilli are also found in the caseous areas, but only in small numbers.

Occasionally a dilated capillary channel, filled with red blood corpuscles, can be found in the most advanced areas of caseation. Even in these situations, therefore, the bacilli have still the opportunity of gaining access to the blood stream.

There are masses of broken-down multinuclear leucocytes amongst the caseous material, but there are nowhere any signs of a recent leucocytic infiltration. There is a well-marked layer of plasma cells surrounding the caseous foci.

Cervical.—There are large irregular tracts of advanced caseation. The rest of the gland tissue is engorged with red blood corpuscles. At the margins of the caseous foci are many large giant cells.

Tubercle bacilli occur, but not in large numbers, throughout the caseous areas and within giant cells.

The caseous areas are packed with the remains of oxyphil leucocytes ; these cells are also very abundant in the rest of the gland. There are many typical plasma cells, and transitional forms are observed between these and what appear to be ordinary large lymphocytes.

Bronchial.—The cortex contains many tubercles, which are caseous, but not to such an advanced degree as those in the glands previously described. Surrounding and near to the tubercles large giant cells occur very abundantly. The rest of the gland, both the medulla and the non-tubercular parts of the cortex, is black with dust particles. There are no deposits of dust within or near the tubercles.

Bacilli are moderately numerous within the tubercles and giant cells, but none are present in the dust-laden areas. This observation, so far as it goes, suggests that the bacilli may not have arrived in the glands by way of the lymph stream from the lungs.

Within some of the tubercles are capillaries containing red corpuscles.

There is only a slight leucocytosis. Many of the giant cells are undoubtedly formed from the endothelial cells lining the trabeculæ. Plasma cells are much fewer than in the more advanced glands described above.

Mesenteric.—The mesenteric gland examined is normal.

SUMMARY.

Generalised tuberculosis. The incidence of the disease is particularly severe on the lungs. The tubercles are caseous and progressive. There is a well-marked cellular reaction, particularly on the part of the multinuclear leucocytes. Bacilli are scanty in number.

VIRUS.—B I.

Animal Inoculated.—Monkey (Rhesus) 2.

Monkey 2 was inoculated subcutaneously in the abdominal wall with an emulsion of the tissues of G.P. 130 and died 41 days afterwards.

Lungs.

General Histological Characters.—In sections taken from two portions of the lungs, isolated areas of consolidated tissue are found. The largest of these areas are about ¼ inch in diameter ; the smallest are very minute. In the consolidated areas there is evidence of necrotic change, but the necrosis is not very advanced ; and even in the most extensive of these areas the distribution of the lung alveoli can be fairly well made out. The general appearance of the consolidated patches differs from that of typical miliary tubercles. They contain no cell structures specially characteristic of tubercles ; the cells are not distributed in zones of different tpyes ; there is no fibrous tissue formation at their periphery. Apart from the consolidated foci, the lung tissue exhibits no histological abnormalities noticeable under a low magnification.

Fibrin.—A few strands of fibrin are found within the consolidated areas. These areas are not surrounded by a peripheral zone of fibrinous deposit.

Minute Characters of Cells.—The cell details are not well brought out. This is perhaps due, to some extent to *post-mortem* change. A few plasma cells are found between the alveoli and surrounding the consolidated areas. In the blood-vessels the white corpuscles appear to be in excess of the normal, and in the consolidated areas there are large numbers of nuclei which probably belong to polymorphonuclear leucocytes, but the staining reaction is not sufficiently good to determine exactly the relative proportion of this type of cell.

Distribution of Bacilli.—Tubercle bacilli are present in enormous numbers, and are aggregated into colonies visible with a low power of the microscope. These clusters of bacilli form conspicuous rings surrounding the consolidated foci ; within these rings the tissue is swarming with isolated bacilli. Many of the colonies seem to have produced little or no tissue reaction at the site of their growth ; the tissue cells amongst which they are growing still retain a nuclear stain, and under a high magnification a cell nucleus is often found with a dense ring of bacilli surrounding it. Amongst the lung alveoli, which, under a low power, appear to be normal, tubercle bacilli are present in large numbers, and seem, from their presence in otherwise normal blood capillaries to have been freely circulating ; they have also surrounded and made their way within a large number of cells of the alveolar epithelium. The presence of large numbers of tubercle bacilli in situations where there is a complete absence of tissue reaction is a striking feature in section of this monkey's lungs.

Characters of Bacilli.—The bacilli vary in length from 2 to 4·5μ, the average being about 3μ. Straight and curved forms are about equally numerous. About three-fourths of the bacilli are beaded or irregularly stained.

Liver.

Histological Changes.—The liver is riddled with necrotic tracts which are very variable in size and outline. In some of them there are the nuclear remains of multinuclear leucocytes, but on the whole there is very little cellular infiltration and there is no evidence of reaction on the part of the fixed tissue cells. No giant cells are present.

Distribution of Bacilli.—Bacilli are present in enormous masses. They form dense clumps in the necrotic foci and are also very abundant in the rest of the tissue. They are numerous in all the blood-capillaries and also in the larger vessels.

Bronchial Gland.

The greater part of the section exhibits semi-necrotic tracts, which are occupied by very large numbers of multinuclear leucocytes. The gland is swarming with bacilli which are aggregated into dense colonies.

Spleen.

The spleen is in a condition of extensive necrosis and is packed with enormous numbers of bacilli.

SUMMARY.

Highly acute infection, characterised by necrotic change with very little tissue reaction, and by the presence of enormous numbers of bacilli, both in the necrotic areas and throughout the vascular system.

VIRUS.—B I.

Animal Inoculated.—Monkey (Rhesus) 4.

Monkey 4 was inoculated intravenously with an emulsion of the tissues of G.P. 130, and died 15 days afterwards.

Lungs.

General Histological Characters.—Sections from three portions of the lungs show that the alveolar walls are thickened, their epithelial cells swollen, and their blood-capillaries engorged. Very little coagulated deposit is present within the alveoli. Small patches of consolidated tissue are found; they are irregular in outline, have no definite fibrous tissue demarcation, and are partially necrotic in the centre.

Fibrin.—No fibrin is demonstrable.

Minute Characters of Cells.—The cells stain badly, especially their protoplasm, and it is impossible to differentiate many of the finer details. Swollen and desquamated epithelial cells are everywhere present and are most conspicuous at the periphery of the consolidated areas. In the interstitial tissue of the alveolar walls there is an increased number of connective tissue corpuscles, and some leucocytic infiltration. Many of the blood-vessels contain a large number of white corpucles, some with irregular or horse-shoe nuclei, some with round nuclei and a large amount of protoplasm; they do not stain well enough for further differentiation. No satisfactory plasma cell reaction can be obtained.

Distribution of Bacilli.—Tubercle bacilli occur in enormous numbers. In all the necrotic patches they are massed together in groups visible under a lower power and occupying the entire area of necrosis; there is no marked tendency for them to accumulate in greater numbers round the periphery of these areas. Tubercle bacilli occur also throughout the tissue in the blood capillaries and within and amongst the cells of the alveolar epithelium; not many are noticeable within the larger blood vessels. The most striking feature about all the sections is the relatively small amount of tissue change; in sections not stained for tubercle bacilli there is nothing to suggest that these are to be found in such enormous numbers.

Characters of Bacilli.—The bacilli vary in length from 2 to $4 \cdot 5\mu$ and average about 3μ. Straight and curved forms are about equally numerous. More than half the bacilli are beaded or irregularly stained.

Liver.

The tissue is permeated with necrotic foci which are smaller, individually, than those found in the liver of Monkey 2, but are more closely set together. Tubercle bacilli are very abundant in all these foci; they also occur throughout the rest of the tissue and are freely circulating in the blood-capillaries.

Bronchial Gland.

The gland examined contains many small semi-necrotic foci which are infiltrated with leucocytes and contain very large numbers of bacilli. Apart from these foci, bacilli are only rarely met with.

Spleen.

The spleen, though crowded with red corpuscles, is relatively poor in nucleated cells. There are many slightly necrotic areas and a few small aggregations of multinuclear leucocytes and their remains. There are no definite tubercles. In every part of the tissue tubercle bacilli are distributed in large numbers.

SUMMARY.

Highly acute infection. Bacilli very abundant both in the tissues and the blood capillaries. Commencing necrosis. No evidence of conservative reaction.

VIRUS.—B I.

Animal Inoculated.—Monkey (Rhesus) 10.

Monkey 10 was inoculated subcutaneously, in the ears, with a culture of G.P. 14, and died 74 days afterwards. The culture used was a primary culture, 57 days old.

Lungs.

General Histological Characters.—Sections have been prepared from four portions of the lungs. Some sections consist of consolidated necrosing tissue similar in character to that found in lobar pneumonia. In other sections the consolidated areas consist of small isolated patches, which are irregular in outline and appear to be gradually extending at the expense of the adjacent alveoli. They have no fibrous tissue boundary. A blood-vessel is generally found either within or in contact with them. The rest of the lung tissue exhibits some compensatory emphysema and some catarrhal change.

Fibrin.—Many of the blood-vessels contain early fibrin. A few small tracts of fibrin are found in various parts of the consolidated tissue.

Minute Characters of Cells.—Plasma cells are abundant and well differentiated; they occur in the neighbourhood of the bronchioles, at the periphery of the consolidated areas, and in the areas of commencing catarrhal change.

Distribution of Bacilli.—In the large areas of pneumonic consolidation, tubercle bacilli are present in moderate numbers. The inflammatory tissue surrounding these areas contains tubercle bacilli in much larger numbers. Many of the bacilli are intracellular; they are frequently found within desquamated cells of the alveolar epithelium. The consolidated foci within the lung substance also contain tubercle bacilli; some of the smaller of these foci are swarming with them. No tubercle bacilli have been noted within the blood-vessels.

Liver.

The liver is crowded with small miliary tubercles. These show partial disintegration of the liver parenchyma, an increase of the endothelial element, and an infiltration with leucocytes and lymphocytes. Bacilli are present in all of them, but only in small numbers; they are not aggregated into groups.

Kidneys.

Immediately beneath the capsule there are a few small patches of interstitial infiltration, associated with partial necrosis of the renal cells. In these situations tubercle bacilli are present in small numbers.

Bronchial Gland.

The gland examined is in a state of advanced necrosis, and contains large numbers of bacilli.

Spleen.

The spleen contains many large tubercles which are extensively caseous, and, where not completely necrotic, contain masses of multinuclear leucocytes and their remains. No giant cells are present. Bacilli are numerous in all these tubercles, particularly at the periphery. Smaller necrotic foci, less rich in bacilli, are also present, and bacilli are to be found in situations which are histologically normal.

SUMMARY.

Progressive disseminated tuberculosis. In the lungs and spleen the lesions are advanced and contain, particularly at their periphery, numerous bacilli. No bacilli have been found within blood-vessels.

VIRUS.—B I.

Animal Inoculated.—Monkey (Rhesus) 18.

Monkey 18 was inoculated intravenously with an emulsion of the spleen of Monkey 20, and was killed, when moribund, 17 days afterwards. Monkey 20 had been intravenously inoculated with an emulsion of the spleen of Monkey 2.

Lungs.

General Histological Characters.—Sections have been prepared from two portions of the lungs. The general characters of the tissue are those of a catarrhal process with occasional small foci of consolidation.

Fibrin.—There are a few small areas containing coagulated material which might be fibrinous; but, on the whole, the presence of demonstrable fibrin is doubtful.

Minute Characters of Cells.—The blood-vessels contain an abnormally large number of leucocytes and lymphocytes. In the bronchioles are found desquamated epithelial cells, polymorphonuclear leucocytes, and red blood corpuscles. The staining reactions of the tissue are not good.

Distribution of Bacilli.—The entire lung tissue is swarming with tubercle bacilli. In all the areas which show any tendency to consolidation, they are so numerous as to be visible with a low power; elsewhere, they are found in and amongst the epithelial cells, in the capillaries, and within the large blood vessels.

Liver.

The tissue is riddled with caseating foci. Multinuclear leucocytes and the nuclear fragments of these cells are abundant in these areas, but other cells have almost completely disappeared. All these foci are swarming with bacilli. Bacilli are also present amongst intact liver tissue, and within blood capillaries.

Bronchial Gland.

The gland examined shows extensive tracts of commencing necrosis, and is swarming with bacilli.

SUMMARY.

Highly acute infection. Disseminated patches of necrosis. Bacilli are present in very large numbers in all the lesions and are also found within the blood-vessels.

VIRUS.—B I.

Animal Fed.—Monkey (Rhesus) 62.

Monkey 62, aged six months, was fed during 66 days, with the milk of Cow 74, and was killed 72 days after the commencement of the experiment.

Lungs.

General Histological Characters.—Sections have been prepared from three portions of the lungs. There are large areas of consolidated tissue, which are almost entirely necrotic except at their periphery; these areas are not bounded by any fibrous tissue zone; many of them merge into the surrounding lung tissue, which is highly œdematous and contains a coagulated deposit in many of the alveoli. In other places there are smaller and more definitely circumscribed consolidated areas. The rest of the lung tissue exhibits acute catarrh, with occasional patches of emphysema. Some of the patches of consolidation are in proximity to bronchi; others are subpleural.

Fibrin.—Within and surrounding the consolidated areas fibrinous deposits are demonstrable.

Minute Characters of Cells.—At the periphery of some of the consolidated areas there is a proliferation of the alveolar epithelial cells, and an infiltration with small round cells, apparently lymphocytes, and a smaller number of multinuclear leucocytes. Plasma cells are not demonstrable.

Distribution of Bacilli.—Tubercle bacilli are found in moderate numbers within the consolidated areas. Apart from these areas, none have been noticed in association with intact or desquamated alveolar or bronchial epithelium.

Liver.

Of two sections taken from different parts of the liver, each contains a few minute tubercles, some of which are caseous and contain giant cells. Rarely, a tubercle bacillus is to be found in one of these situations.

Kidneys.

In sections taken from two different situations there are no tubercular lesions and no bacilli. There is a patch of cellular infiltration in one of them.

SUMMARY.

The lungs are very severely affected; the liver slightly. The possibility must be borne in mind of inhalation infection from dried particles of food and excreta in the monkey's cage.

VIRUS.—B I.

Animal Fed.—Monkey (Rhesus) 70.

Monkey 70 (aged six months) was fed during 5 days with the milk of Cow 74, then very rich in tubercle bacilli, and was killed 24 days afterwards.

Lungs.

The lung tissue contains numerous, large, caseous tracts, which ramify in all directions into the surrounding tissue. Between these areas the alveoli show in some places a catarrhal condition and in others compensatory emphysema. The general appearance of the tissue is typical of the tubercular type of pneumonia. The caseous areas and their environments are packed with multinuclear leucocytes and their remains. There is very little evidence of fibrous tissue formation. Tubercle bacilli are present in the lesions in very large numbers

SUMMARY.

Rapidly progressive tuberculosis.

VIRUS.—B I.

Animal Fed.—Monkey (Rhesus) 76.

Monkey 76 (young adult) was fed with ·01 mg. of culture isolated from Cow 44, and was killed 228 days afterwards, when in a moribund condition.

Lungs.

The specimen examined contains four or five tubercles of from $\frac{1}{2}$ to 1 mm. in diameter. The tissue surrounding the tubercles is normal. The tubercles are in different stages of development. One of them is markedly caseous, another is slightly caseous, and the rest are not caseous at all. The first mentioned contains very few bacilli; in the others bacilli are numerous.

Liver.

The liver contains numerous discrete tubercles of various sizes. The larger are extensively caseous, irregular in outline, and though showing some attempt at a fibrous tissue reaction at their periphery, are evidently extending; they contain numerous bacilli, especially in their outer half. Of the smaller tubercles some are caseous and some are not; they contain much fewer bacilli, relatively to their size, than the larger ones.

Kidneys.

The specimen examined contains several discrete tubercles of various sizes. The larger are just commencing to caseate. Bacilli are present in all of them and are numerous in the larger.

SUMMARY.

Disseminated tuberculosis, very slowly progressive. The tubercles contain numerous bacilli.

VIRUS.—B I.

Animal Fed.—Orang 4.

Orang 4 (3 years old) was fed during 12 days with the milk of Cow 44 and died of gastro-enteritis, 16 days after the commencement of the experiment.

Lungs.

The specimen examined shows slight patches of congestion, but nothing histologically suggestive of tuberculosis. No tubercle bacilli have been found in it, but various other bacteria are present.

Mesenteric Gland.

In the gland examined there are no histological abnormalities. No tubercle bacilli have been found in it.

SUMMARY.

No evidence of tuberculosis.

VIRUS.—B IV.

Animal Fed.—Baboon 20.

Baboon 20 (young) was fed during 25 days with 2 litres of milk from Cow 172, and was killed 85 days after the commencement of the experiment.

Lungs.

The specimen of lung is in a semi-solid condition, owing to the presence of many partially confluent, caseating tubercles. The tubercles are in various stages of advance and appear to be progressive. Many giant cells are present at their margins. Tubercle bacilli are present but are not numerous.

Liver.

The liver contains numerous small, miliary tubercles many of which have not begun to caseate. They are infiltrated with leucocytes and lymphocytes and contain giant cells. Tubercle bacilli are present, but rare.

SUMMARY.

Progressive tuberculosis, with severe incidence on the lungs; bacilli not numerous.

VIRUS.—B IV.

Animal Inoculated.—Baboon 46.

Baboon 46 (young) was inoculated subcutaneously with 1 mg. of culture of B IV., and died 49 days afterwards.

Lungs.

Isolated, circumscribed, caseous tubercles are present, containing very few tubercle bacilli.

Kidneys.

A small, doubtful lesion, not containing tubercle bacilli, has been found in the specimen examined.

SUMMARY.

A few caseous tubercles in the lungs, chronic in type and containing very few bacilli.

VIRUS.—B IV.

Animal Fed.—Baboon 62.

Baboon 62 was fed with ·1 mg. of culture of B IV., and died 136 days afterwards.

Lungs.

The tissue is almost completely consolidated and contains many patches of advanced caseation. These patches are not sharply marked off from the rest of the tissue. At their periphery there are many fibroblasts, but these cells do not form a definite layer; they are intermingled with desquamated epithelial cells, and there is a gradual transition from this region to non-caseous areas consisting of recognisable alveoli filled with desquamated epithelium. Giant cells are fairly numerous. Multinuclear leucocytes are plentiful, particularly in the regions which are at the commencement of necrotic change. Tubercle bacilli occur, but only in small numbers, both in the caseous areas and in the rest of the tissue.

Liver.

The liver contains both large, circumscribed, caseous tubercles and many minute tubercular foci which show no sign of caseation. Tubercle bacilli are present, but very rare.

Kidneys.

In the specimen of kidney there is a small amount of infiltration which is not histologically suggestive of tuberculosis. No tubercle bacilli have been found.

Bronchial Gland.

A bronchial gland examined shows small foci which are not caseous but contain large numbers of multinuclear leucocytes. In these foci tubercle bacilli are present in small numbers.

SUMMARY.

Disseminated tuberculosis of a slowly progressive character, with marked incidence on the lungs. Bacilli present in all the lesions, but only in small numbers.

VIRUS.—B IV.

Animal Fed.—Chimpanzee 2.

Chimpanzee 2 (young) was fed with 1 mg. of culture of B IV., and died 56 days afterwards.

Lungs.

Histological Changes.—The tissue examined contains two caseous tubercles, each about 1 mm. in diameter, and several smaller caseous areas of irregular outline. The tubercular foci are not definitely circumscribed, but appear to be spreading and becoming confluent. The adjacent lung tissue shows marked thickening and infiltration of the alveolar walls, and many of the alveoli are partially collapsed. The tissue as a whole appears to be slightly necrotic, and does not react well to differential stains.

Distribution and Characters of Bacilli.—Bacilli are fairly numerous in all the caseous areas. Most of them are straight or only slightly curved, and about $2 \cdot 5\mu$ in length. Many of them are partially beaded.

Mesenteric Glands.

The mesenteric gland examined is caseous almost throughout, and contains large numbers of bacilli.

Intestine.

In a section through a tubercular ulcer of the small intestine bacilli are present, but can only be found with difficulty.

SUMMARY.

Progressive tuberculosis of the lungs.

VIRUS.—B IV.

Animal Inoculated.—Chimpanzee 4.

Chimpanzee 4 (young) was inoculated subcutaneously over the right scapula with 1 mg. of culture of B IV., and died 55 days afterwards.

Lungs.

Histological Changes.—The lung is packed with miliary tubercles, the larger of which are at the commencement of caseation. The tubercles are not sharply marked off from the rest of the tissue, but appear to be extending in all directions, and in some situations are confluent. The surrounding tissue shows desquamated epithelium, compressed alveoli, and thickened alveolar walls. Lymphocytes are almost as numerous as leucocytes, except in the largest tubercles, which contain masses of broken down multinuclear leucocytes. There are groups of plasma cells at the periphery of many of the tubercles. No fibrin is demonstrable.

Distribution and Characters of Bacilli.—In all the definitely tubercular areas and in some of the thickened alveolar walls bacilli are present. In many situations they are very numerous, and sometimes they occur in groups. There are many short and straight forms, measuring from 1 to $1 \cdot 5\mu$, and also a somewhat smaller number of longer forms, measuring from $2 \cdot 5$ to $3 \cdot 5\mu$. These longer forms are often curved and sometimes beaded.

Liver.

Histological Changes.—The liver is packed with very small, discrete, miliary tubercles, several appearing in every microscopic field, under a low power. The larger of these tubercles are caseous and are more extensively infiltrated with multinuclear leucocytes than the smaller. In some of the larger tubercles there is a fibrinous exudate.

Distribution of Bacilli.—Bacilli are rare in the smallest tubercles, but numerous in some of the larger.

Kidneys.

Histological Changes.—In the section examined there is a miliary tubercle about 1 mm. in diameter immediately beneath the capsule, and a few smaller tubercles are found rather deeper in the substance of the cortex. There is a slight general increase of the interstitial tissue. Plasma cells are present at the margin of the tubercular areas.

Distribution of Bacilli.—Bacilli are present in some of the lesions, but are rare.

Heart Muscle.

In the section of the heart muscle there is a small nodule characterised by an increase in the endothelial cells and an infiltration of lymphocytes and leucocytes. In this area a few tubercle bacilli are present.

Axillary Gland.

The gland examined is in a condition of advanced necrosis, and contains bacilli in small numbers.

Portal Gland.

Contains large caseous tracts in which bacilli are present but rare.

SUMMARY.

Acute, disseminated tuberculosis, the incidence of the disease being particularly severe on the lungs.

VIRUS.—B IV.

Animal Fed.—Chimpanzee 6.

Chimpanzee 6 (young) was fed during one week with one litre of milk from Cow 172, containing approximately 100,000,000 bacilli, and was killed 100 days after the commencement of the experiment.

Lungs.

Histological Changes.—The tissue examined shows general thickening and infiltration of the alveolar walls and small patches of consolidation which have no definite outline, but merge into the surrounding tissue. Some of these areas are slightly caseous. No fibrin is demonstrable.

Distribution and Characters of Bacilli.—Bacilli are numerous in the consolidated areas; in some cases they are so plentiful as to be just visible with a low power. They vary in length from 1·5 to 4μ. The shorter forms are straight and uniformly stained; the longer forms which are quite as numerous as the shorter, are slender, generally curved, and frequently beaded.

Liver.

No tubercles and no bacilli found.

Kidneys.

There is some interstitial infiltration, but no definite tubercular lesions and no bacilli have been found.

Mesenteric and Colic Glands.

A mesenteric and a colic gland examined are in a condition of advanced caseation and contain numerous bacilli.

SUMMARY.

Progressive tuberculosis of the lungs, with numerous bacilli.

VIRUS.—B IV.

Animal Fed.—Chimpanzee 8.

Chimpanzee 8 (young) was fed with 10,000,000 bacilli contained in the milk of Cow 172 and died 144 days afterwards.

Lungs.

The tissue contains numerous patches of consolidation which are filled with multinuclear leucocytes and do not resemble tubercular lesions. No tubercle bacilli are present.

Bronchial Gland.

The bronchial gland examined contains no histological evidence of tuberculosis and no tubercle bacilli have been found in it.

Mesenteric Gland.

A small mesenteric gland examined is normal.

SUMMARY.

The absence of tubercle bacilli and of tubercles in the lungs is in marked contrast to the condition of Chimpanzee 6. According to the post-mortem notes, death appears to have been due to pneumococcal broncho-pneumonia.

VIRUS.—B IV.

Animal Fed.—Chimpanzee 10.

Chimpanzee 10 was fed with ·1 mg. of a culture of B IV., isolated from Chimpanzee 8, and died 113 days afterwards.

Lungs.

The tissue is consolidated and contains large, irregular, caseous areas, which show no sign of demarcation into definite tubercles. In the regions not completely necrotic there is a dense infiltration of multinuclear leucocytes. No giant cells have been found. There are some small patches of material which is recognisable as fibrin, and there is a good deal of deposit which is probably old fibrin. Bacilli are present everywhere, and in some situations are very numerous. Their length varies from 1 to 2·5μ, the average being about 2μ. The majority are straight.

Liver.

Miliary tubercles are numerous. They contain many giant cells and are generally caseous. Small lymphocytes are generally abundant at the margin of these areas. Tubercle bacilli are present in scanty numbers.

Kidneys.

There is some interstitial infiltration, but neither tubercles nor tubercle bacilli have been found.

Lymphatic Glands.

Cervical.—A cervical gland contains large tracts of advanced caseation and also many smaller foci where caseation is just commencing. Bacilli are present in all the foci and are moderately numerous in some.

Bronchial.—A small bronchial gland contains early tubercles in which bacilli are present in small numbers.

Mesenteric.—A mesenteric gland is very extensively affected and contains numerous bacilli; they form groups just visible under a low power. No fibrin is demonstrable.

Ileo-Colic.—In the gland examined the tissue is entirely replaced by non-vascular, necrotic material. Bacilli are fairly plentiful throughout, but nowhere form dense masses.

SUMMARY.

Progressive, disseminated tuberculosis, with a particularly severe incidence on the lungs. Bacilli are numerous in the lungs and mesenteric glands but less numerous elsewhere.

VIRUS.—B IV.

Animal Fed.—Chimpanzee 16.

Chimpanzee 16 was fed with ·01 mg. of culture from Chimpanzee 10, and died 91 days afterwards.

Lungs.

The lungs are in a catarrhal condition. Most of the alveoli are filled with a coagulated exudate, and there is much desquamation of epithelial cells. Large patches of tubercular consolidation are also present. These are partially caseous; they are not sharply marked off from the rest of the tissue, but show a tendency to fibroid transformation. They contain numerous tubercle bacilli. The bacilli vary in length from 1·5 to 3μ, the average being about 2μ. The majority are straight.

Liver.

The liver is in a condition of fatty degeneration. It contains a large number of miliary tubercles, which, like the lesions in the lungs, show a tendency to fibroid transformation. Many of them are caseous. Most of the tubercles contain several bacilli.

Kidneys.

Caseating tubercles are present beneath the capsule and in the interior of the cortex. The tubercles are not sharply marked off from the rest of the tissue. Bacilli are present in small numbers.

Portal Gland.

Large caseous areas are present. Bacilli are particularly numerous at the periphery of these areas and are evidently invading the normal tissue. A great many of the bacilli are intracellular. The tubercles show no sign of conservative change.

SUMMARY.

Disseminated tubereulosis. The lesions in the lungs and liver show an effort at tissue resistance, but are evidently progressive.

VIRUS.—B IX.

Animal Fed.—Chimpanzee 14.

Chimpanzee 14 was fed with 1 mg. of culture of B IX., and died 63 days afterwards.

Lungs.

Isolated tubercular foci are frequent. The smallest are not more than the size of an alveolus; the largest are from 1 to 2 mm. in diameter. Continuous with the larger there is an infiltration of the walls of adjacent alveoli. The tubercles are in an early stage of formation, even the largest being only slightly caseous; they are not bounded by any definite zone, but merge into the adjacent tissue. They all contain tubercle bacilli, and in the larger, bacilli are fairly numerous. Apart from these tubercular foci, there are some areas of partial collapse, but otherwise the lung tissue is normal. The amount of normal tissue is much in excess of that which is definitely tubercular. The bacilli vary in length from 1·5 to 3μ, the average being about 2μ. Most of them are straight.

Liver.

Small miliary tubercles, centrally caseous, and surrounded with a zone of leucocytes and lymphocytes, occur frequently in the liver substance. A small fibroid tubercle, forming a flattened projection beneath the capsule, has also been noted. Bacilli are present, but rare.

Kidneys.

A few suspicious foci have been found, but no bacilli have been demonstrated in them.

Mesenteric Gland.

The gland examined is highly necrotic, and contains numerous bacilli.

SUMMARY.

Disseminated tuberculosis, with early, progressive lesions in the lungs. The disease in the lungs is not sufficiently extensive to account for death.

VIRUS.—B IX.

Animal Fed.—Chimpanzee 18.

Chimpanzee 18 was fed with ·01 mg. of culture of Chimpanzee 14, and died 116 days afterwards.

Lungs.

The tissue is in a catarrhal condition and contains numerous patches of consolidation which are irregular in outline and are commencing to caseate. Fibrin is present, particularly in these areas.

Tubercle bacilli are moderately numerous and are notably short and straight. No large groups have been found.

Liver.

Tubercles of various sizes, some large and some minute, are abundantly distributed throughout the tissue. The lesions have no sharply defined boundary zone. The largest are extensively caseous. They all contain numer-

ous miltinuclear leucocytes. Fibrin is present in both the larger and the smaller tubercles.

Bacilli are fairly abundant in the large tubercles, but are not numerous in the smaller. In addition to short and straight forms, occasionally longer and curved forms are seen.

Kidneys.

Small caseating tubercles, with no definite boundary zone, are present in the cortex. They are infiltrated with multinuclear leucocytes. Bacilli are not very numerous.

Mesenteric Gland.

The gland examined is almost completely caseous and contains the remains of large numbers of multinuclear leucocytes. Bacilli are abundant.

SUMMARY.

Acute generalised tuberculosis.

VIRUS.—B IX.

Animal Fed.—Lemur 2.

Lemur 2 was fed with 1 mg. of culture of B IX., and was killed 46 days afterwards.

Lungs.

The lungs contain large tubercles in an advanced stage of caseation. These tubercles are not surrounded by fibrous tissue. The alveoli immediately adjacent to them are partly collapsed and partly consolidated. The tissue more remote from these tubercles is to a large extent normal, but there are some areas of compensatory emphysema. Bacilli are present in the tubercles, but are rather scanty.

Liver.

The liver contains many small caseous tubercles, which are frequently surrounded by a zone of lymphocytes and leucocytes. The tubercles contain very few bacilli.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

SUMMARY.

Disseminated tuberculosis. The lesions in the lungs and liver are of a chronic type, but are not definitely retrogressive. Bacilli are scanty.

VIRUS.—B IX.

Animal Inoculated.—Lemur 10.

Lemur 10 was inoculated subcutaneously with ·01 mg. of culture of B IX., isolated from Lemur 2, and died 30 days afterwards.

Lungs.

The specimen shows a tubercle which is slightly necrotic and is packed with multinuclear leucocytes. It possesses no fibrous boundary. In the surrounding tissue the blood capillaries are congested and there is a thickening of the alveolar walls. The tubercle contains numerous bacilli, some of which are enclosed within multinuclear leucocytes.

Liver.

The liver contains many minute, caseating foci in which multinuclear leucocytes are very abundant. Bacilli are present in these foci, but are much less numerous than in the tubercle in the lungs described above.

Kidney.

No tubercles or bacilli have been found.

SUMMARY.

The tubercles in the lung are not numerous. In the liver they are more numerous, but smaller, and contain fewer bacilli. In both lungs and liver the bacilli are being vigorously attacked by the leucocytes.

VIRUS.—B IX.

Animal Inoculated.—Lemur 12.

Lemur 12 was inoculated subcutaneously with 1 mg. of culture of B IX., isolated from Lemur 2, and was killed 23 days afterwards.

Lungs.

Two specimens, from different parts of the lung, have been examined and found to be normal.

Liver.

Throughout the liver substance are large numbers of small, miliary tubercles. These tubercles are slightly necrotic and contain large numbers of leucocytes. They have no fibrous or other cellular boundary. Early fibrin is demonstrable in nearly all of them. They all contain large numbers of bacilli.

Kidneys.

No tubercles have been found. Here and there, there is a very slightly increase in the elements of the interstitial tissue. The increase is so slight that it hardly seems sufficient to term pathological. In these situations however, tubercle bacilli are present.

Cervical Gland.

A cervical gland examined is overrun with large numbers of bacilli. The areas in which the bacilli are most numerous have partially lost their protoplasmic staining properties but are not caseous. They contain large numbers of multinuclear leucocytes. No fibrin is demonstrable.

SUMMARY.

The lung appears to have escaped infection. The liver is in a condition of acute miliary tuberculosis and contains large numbers of bacilli. The kidneys show an early invasion of bacilli, without marked tissue change. The cervical gland is overrun with bacilli, but shows only a slight amount of tissue destruction.

VIRUS.—B IX.

Animal Fed.—Mandrill Baboon 54.

Mandrill Baboon 54 (young) was fed with 1 mg. of culture of B IX., and was killed 49 days afterwards.

Lungs.

In the specimen examined, the tissue is normal with the exception of a consolidated patch measuring about 3 mm. in length by 1 mm. in breadth. This patch is very irregular in outline and appears to be extending. In the older portion of it the alveolar structure is completely obliterated and there is commencing caseation; elsewhere, alveoli, filled with desquamated epithelial cells, are recognisable. Giant cells, and fusions of epithelial cells which resemble giant cells are frequent. This consolidated area contains large aggregations of both lymphocytes and leucocytes. Bacilli are scantily distributed, but occasionally form small groups. The masses of leucocytes appear to have a definite association with these groups; the lymphocytes occur in greatest abundance where bacill are rare.

Liver and Kidneys.

These organs contain some rather suspicious patches of infiltration, but no definite tubercles and no bacilli have been found.

Cervical Gland.

The gland examined contains a few early foci in which bacilli occur in small numbers.

Summary.

Slight tuberculosis of the lungs.

VIRUS.—B IX.

Animal Fed.—Mangabey 2.

Mangabey 2 (young) was fed with 1 mg. of culture of B IX., and was killed 47 days afterwards.

Lungs.

In one specimen of lung neither tubercles nor tubercle bacilli have been found. In a second specimen there is a large pneumonic patch which is partially caseous and contains in its less necrotic portions several giant cells. Tubercle bacilli are moderately numerous in the consolidated area.

Liver.

In one specimen no lesions or bacilli are present. In a second specimen there is one early tubercle which is not necrotic, but is overrun with lymphocytes and leucocytes though the cells of the liver parenchyma are not obliterated. There is no peripheral increase of fibrous elements. The tubercle contains a few giant cells; in one of these a tubercle bacillus is present.

Kidneys.

No lesions or bacilli have been found.

Mesenteric Gland.

The gland examined exhibits numerous areas of very slight necrosis in which large giant cells are abundant. All these areas contain many bacilli; the bacilli are particularly numerous within the giant cells.

Spleen.

No lesions or bacilli have been found.

Summary.

The mesenteric gland shows slight tissue destruction and contains numerous bacilli. There is an isolated patch of consolidation in the lung which is partially caseous and contains moderate numbers of bacilli. A small tubercle has been found in the liver.

VIRUS.—B IX.

Animal Fed.—Mangabey 4.

Mangabey 4 was fed with 1 mg. of culture of B IX., and was killed 84 days afterwards.

Lungs.

Histological Changes.—The tissue contains many small tubercles which are slightly caseous, irregular in outline, and not sharply marked off from the rest of the tissue. Apart from these tubercles many of the alveolar walls are thickened and some of the alveoli are filled with desquamated epithelium. A deposit of fibrin is present in some of the tubercles.

Distribution of Bacilli.—Bacilli are fairly numerous in most of the lesions, particularly in those which are definitely caseous.

Liver.

The liver is crowded with miliary tubercles which are in various stages of caseation. They are not bounded by a definite cellular zone. Some of the tubercles contain a little fibrinous deposit. Bacilli are moderately numerous.

Kidneys.

No tubercles or bacilli have been found in the specimen of kidney.

Lumbar Gland.

The gland examined contains many caseous foci. Bacilli are very numerous and often form conspicuous groups and colonies.

Summary.

Acute, progressive tuberculosis.

VIRUS.—B IX.

Animal Fed.—Monkey (Rhesus) 108.

Monkey 108 was fed with 1 mg. of culture of B IX. and was killed, when moribund, 33 days afterwards.

Lungs, Liver, and Kidneys.

No tubercles or tubercle bacilli have been found in the specimens of these organs.

Mesenteric Gland.

The gland examined contains extensive, semi caseous areas and is swarming with tubercle bacilli.

SUMMARY.

Mesenteric glands acutely infected. No evidence of tuberculosis found in lungs, liver, or kidneys.

VIRUS.—B IX.

Animal Fed.—Monkey (Rhesus) 110.

Monkey 110 was fed with 1 mg. of culture of B IX., and was killed, when moribund, 80 days afterwards.

Lungs.

Histological Changes.—The tissue is in a semi-solid condition, the areas of complete consolidation being patchy in distribution, irregular in outline, and more or less confluent at their periphery. Some of these areas are slightly caseous in the centre; they are all filled with dense masses of leucocytes and show a marginal infiltration with lymphocytes. Small deposits of fibrin are occasionally met with.

Distribution of Bacilli.—The consolidated tissue is packed with enormous numbers of tubercle bacilli which everywhere form dense aggregations visible under a low power. Bacilli are also distributed in small groups amongst the walls and within the epithelial cells of patent alveoli.

Liver.

Small miliary tubercles, slightly necrotic and containing many bacilli, are scattered in large numbers throughout the tissue. Bacilli also occur within the blood capillaries. Some fibrin is present.

Kidneys.

In both cortex and medulla there are many caseous lesions, all containing bacilli in very large numbers. Multinuclear leucocytes are very abundant in association with the bacilli. Some of the lesions contain a deposit of a doubtful nature, but little or no definite fibrin is present.

Bronchial Gland.

The gland examined, which did not show naked-eye tubercles, contains minute foci of commencing caseation. These contain large numbers of bacilli. Bacilli are also present throughout the rest of the tissue.

SUMMARY.

Acute progressive tuberculosis, associated with enormous numbers of bacilli.

VIRUS.—B IX.

Animal Inoculated.—Monkey (Rhesus) 114.

Monkey 114 was inoculated subcutaneously with ·01 mg. of culture of B IX., isolated from Monkey 110, and died 34 days afterwards.

Lungs.

The tissue is in a condition of nearly complete consolidation. A few tubercles in an early stage of caseation are present, but the greater part of the tissue is not typical of tubercular pneumonia. Bacilli are present in moderate numbers within the tubercles, and are occasionally found in other parts of the tissue.

Liver.

The liver is crowded with miliary tubercles. The largest are in an advanced stage of caseation; the smallest have hardly begun to caseate. They contain an abundant deposit of fibrin. Bacilli are fairly numerous in all the tubercles, and also occur in the rest of the tissue.

Kidneys.

Caseous tubercles, containing many bacilli, are present in the cortex.

Axillary Gland.

The gland is in a condition of very advanced caseation and contains numerous bacilli.

SUMMARY.

Disseminated tuberculosis with particularly severe incidence on the liver.

VIRUS.—B IX.

Animal Inoculated.—Monkey (Rhesus) 116.

Monkey 116 was inoculated subcutaneously with 1 mg. of a culture of B IX., isolated from Monkey 110, and died 36 days afterwards.

Lungs.

The specimen contains some caseous tubercles which are invaded with large numbers of leucocytes and contain many bacilli. The surrounding tissue is congested, but contains no lesions associated with tubercle bacilli Some deposits of fibrin occur in the tubercles.

Liver.

Small miliary tubercles are plentiful. They contain large numbers of bacilli and show a little fibrinous deposit.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

SUMMARY.

The disease in the lungs and liver is as severe as that produced in Monkey 114.

VIRUS.—B IX.

Animal Fed.—Monkey (Rhesus) 130.

Monkey 130 was fed with ·01 mg. of culture from Monkey 116 and was killed 86 days afterwards.

Lungs.

The specimen of lung is completely consolidated and contains large, irregular, caseous tracts. Tubercle bacilli are present throughout the tissue, but nowhere form large groups.

Liver.

In the specimen of liver no lesions or bacilli have been found.

Kidneys.

The specimen contains a caseous tubercle, in which bacilli are scanty.

Bronchial Gland.

The gland contains many caseous foci. Bacilli are scanty.

SUMMARY.

The lungs are in a condition of advanced tubercular pneumonia.

VIRUS.—B XXVI.

Animal Inoculated.—Monkey (Rhesus) 142.

Monkey 142 was inoculated subcutaneously with ·001 mg. of culture of B XXVI., and died 45 days afterwards.

Lungs.

Histological Changes.—The tissue is congested and contains small caseous tubercles of irregular outline, which are not bounded by any definite peripheral zone, but merge into the surrounding tissue.

Distribution of Bacilli.—These tubercles are crowded with colonies of bacilli. Bacilli are also very abundant in many other parts of the tissue, where no definite tubercles have been formed. The bacilli vary in length from 2 to 4·5 and are for the most part curved and uniformly stained.

Liver.

The liver is riddled with caseating tubercles, both large and small. The larger are almost completely necrotic. The tubercles have no peripheral zone, but are obviously progressive. They are all crowded with tubercle bacilli. Bacilli are also present in the intact liver tissue.

Kidneys.

Small, caseous tubercles with no definite peripheral zone are present in the cortex. They are swarming with bacilli. No bacilli have been found apart from these lesions.

Bronchial Gland.

The gland is almost completely caseous and is swarming with bacilli.

SUMMARY.

Acute, disseminated tuberculosis, with enormous numbers of bacilli. It is noteworthy that the dose inoculated was very small.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 1. C.M.

Animal Inoculated.—Monkey (Rhesus) 1.

Monkey 1 was inoculated subcutaneously with an emulsion of tissues from G.P.s 108 and 111, and was killed, when moribund, 52 days afterwards.

Lungs.

General Histological Characters.—Sections taken from two portions of the lungs show numerous miliary tubercles. The larger of these are about the size of the microscopic field under a low magnification (Zeiss's 16 mm. Apochromatic Objective; Compensating Eye Piece No. 4). In many places two or more tubercles partially coalesce. The larger tubercles are surrounded by a narrow but fairly well defined fibrous tissue zone, and are almost completely necrotic in their centre. The smaller tubercles show less necrosis, and have little or no fibrous tissue zone. The rest of the lung tissue exhibits a catarrhal condition. The alveolar walls are thickened, the blood capillaries are engorged, and many of the alveoli contain a coagulated exudate and desquamated epithelial cells.

Fibrin.—No fibrin is demonstrable by either Kochel's or Weigert's method, or in eosin and methylene blue specimens.

Minute Characters of Cells.—In the central portion of the tubercles there is no evidence of cell proliferation or infiltration. In the earlier tubercles desquamated epithelial cells are still to be found, but there is no sign of newly formed cells of "epithelioid" type. No giant cells occur in any of the tubercles. Plasma cells occur around the margin of the nodules, and in the walls of the alveoli; a ring of these cells is also to be found round many of the bronchioles. The blood vessels contain numerous white corpuscles, both lymphocytes and multinuclear leucocytes; very few cells of the latter type can be demonstrated in other situations. All the nodules contain a good deal of nuclear staining material which is too much disintegrated to justify an opinion as to the characters of the cells from which it has been derived.

Distribution of Bacilli.—Numerous tubercle bacilli are scattered throughout the tubercular nodules, but they do not form such dense masses as to be visible under a low magnification. Tubercle bacilli are also found in the rest of the lung tissue, but in much smaller numbers.

Characters of Bacilli.—The bacilli vary in length from 2 to 5μ, the average being over 3μ. Nearly all the bacilli are beaded, or show a tendency to beading. The beading is more marked amongst the bacilli which occur in the necrotic areas.

Liver.

The tissue is riddled with large, irregular, caseous tracts. Where not completely necrotic, these contain large numbers of leucocytes. At their periphery, which is not sharply defined, lymphocytes are also present, but there is no evidence of fibrous tissue formation. Bacilli are extremely numerous, and in some of the necrotic foci form groups of colonies. The tubercles are evidently extending, and their periphery is richly supplied with bacilli. As in the lung, the bacilli are notably long.

Kidneys.

Large tubercles very rich in bacilli and in an advanced stage of caseation are present. The tubercles are not sharply marked off from the rest of the tissue.

Mesenteric Glands.

The gland is completely caseous and is swarming with bacilli.

Spleen.

The spleen is in an advanced stage of caseation and contains enormous numbers of bacilli.

SUMMARY.

Highly acute infection, characterised by rapidly progressive necrosis and the presence of enormous numbers of bacilli. No giant cells are present. The bacilli are notably long.

VIRUS.—H 1. C.M.

Animal Inoculated.—Monkey (Rhesus) 3.

Monkey 3 was inoculated subcutaneously with culture isolated from G.P. 4, and was killed 34 days afterwards.

Lungs.

General Histological Characters.—Sections have been prepared from four portions of the lungs. I have found a few small isolated foci of consolidation, which are in a condition of partial necrosis, and are surrounded by a fibrous tissue zone. The rest of the lung tissue contains some catarrhal patches, and a few emphysematous areas, but is otherwise normal.

Fibrin.—No fibrinous deposits are demonstrable.

Minute Characters of Cells.—A few plasma cells are demonstrable in the areas of congestion. The cells within the fibrous tissue zone of the necrotic patches are too degenerate to be differentiated.

Distribution of Bacilli.—The tubercle bacilli are confined to the necrotic patches and their immediate environment. Within the necrotic areas they occur in moderately large numbers; in the connective tissue environment they occur rather more scantily and are often intracellular.

Characters of Bacilli.—The bacilli vary in length from 2 to 5μ, averaging about $3{\cdot}5\mu$. The majority are curved. Beaded and uniformly stained forms are about equally numerous.

Liver.

The lesions are similar to those found in the liver of Monkey 1, but of rather smaller size. Bacilli, though plentiful, are less abundant than in the liver of Monkey 1, and have not grown into clumps.

Kidneys.

No lesions or bacilli have been found in the specimen examined.

Portal Gland.

Advanced caseation. Bacilli very numerous.

Spleen.

The spleen is in a condition of advanced caseation. Bacilli are extremely abundant, and in many cases have aggregated into groups of colonies.

SUMMARY.

Similar to Monkey 1.

VIRUS.—H 1. C.M.

Animal Inoculated.—Monkey (Rhesus) 7.

Monkey 7 was inoculated subcutaneously into both ears and both hind legs with a culture from G.P. 51. The animal died 20 days afterwards.

Lungs.

General Histological Characters.—Sections prepared from two portions of the lungs give general evidence of catarrh, and exhibit small irregular foci of consolidation and necrosis without the formation of typical tubercles.

Fibrin.—No fibrin is demonstrable.

Minute Characters of Cells.—Many of the detached and swollen epithelial cells have begun to proliferate in small groups. The alveolar walls are infiltrated with small cells, many of which are leucocytes. Some of the bronchioles contain red blood corpuscles in addition to desquamated epithelial cells. The tissues do not stain well enough for the demonstration of finer details.

Distribution of Bacilli.—Tubercle bacilli are numerous in the areas of consolidation, and occur, though in smaller number, throughout the rest of the tissues. Many of them are contained within epithelial cells. The tissue is swarming with cocci, diplococci, and short bacilli which are not tubercle bacilli. No one type of these extraneous organisms is predominant.

Liver.

The liver is permeated with necrotic areas containing numerous tubercle bacilli. As in the lungs, other organisms are also present.

Kidneys.

A few caseous tubercles, containing large numbers of bacilli, are present. The bacilli are notably long.

Spleen.

The spleen shows diffuse caseation, without the formation of definite tubercles. Bacilli are very abundant, and are becoming massed together into colonies

SUMMARY.

Similai to Monkeys 1 and 3.

VIRUS.—H 1. C.M.

Animal Inoculated.—Monkey (Rhesus) 9.

Monkey 9 was inoculated subcutaneously with an emulsion from the tissues of Monkey 3, and died 35 days afterwards.

Lungs.

General Histological Characters.—Sections have been examined from three portions of the lungs. The lung tissue is somewhat emphysematous. There is very little catarrhal change. Situated just beneath the pleura is an occasional round nodule of necrotic tissue about half the microscopic field (low power) in size, and bounded by a well-marked fibrous tissue zone. In the substance of the lung are a few very small consolidated patches without the structure of an organised tubercle.

Fibrin.—No fibrin is demonstrable.

Minute Characters of Cells.—In the outer and less necrotic part of the subpleural nodules the cells are of the "epithelioid" type, and there are a few cell fusions, which are suggestive but not typical of giant cells. No plasma cells are demonstrable. The blood-vessels contain leucocytes and lymphocytes in abnormally large numbers.

Distribution of Bacilli.—In some of the subpleural nodules tubercle bacilli are aggregated in large numbers visible under a low power. Tubercle bacilli are also present in the foci of consolidation found within the lung substance; in the earlier of these foci they are frequently found within desquamated epithelial cells. In the areas of lung tissue which appear histologically normal tubercle bacilli are found here and there in the blood capillaries and within or closely applied to the normal flattened cells of the lung epithelium. Within the larger blood-vessels, also, tubercle bacilli are found; sometimes they are lying free, more frequently they are within or closely applied to white corpuscles, both those with an irregular nucleus and those with a rounded nucleus and a large amount of protoplasm. Tubercle bacilli are also present in the desquamated epithelial cells found within some of the bronchioles. Although these lungs contain a much smaller number of bacilli than some of those recorded above, the relatively small amount of tissue reaction in proportion to the number of bacilli present is again a striking feature.

Liver.

Miliary tubercles, completely necrotic except for the presence of multinuclear leucocytes and their remains, are distributed throughout the tissue. These contain dense masses of bacilli, forming aggregations visible under a low power. Bacilli are also present in the blood-capillaries and in many parts of the tissue where there are no histological lesions.

Bronchial Gland.

The gland examined contains large caseous areas. These are swarming with bacilli, which are often aggregated into conspicuous colonies.

Spleen.

The spleen is extensively caseous and is swarming with bacilli, which have often grown into colonies. When colonies are formed, the bacilli tend to become shorter than those which occur singly.

SUMMARY.

Highly acute infection, characterised by the rapid multiplication of bacilli and their presence in large numbers in the blood stream. The amount of tissue destruction in the lungs is small relatively to the large number of bacilli present.

VIRUS.—H 8. S.C.

Animal Inoculated.—Monkey (Rhesus) 17.

Monkey 17 was inoculated subcutaneously with an emulsion of original material (human mesenteric glands) and died 68 days afterwards.

Lungs.

General Histological Characters.—Sections have been prepared from three portions of the lungs. Some sections are almost entirely occupied by consolidated necrotic tissue and are typical of lobar pneumonia. In other sections there are patches of intense vascular engorgement, and the alveoli are filled with a coagulated exudate.

Fibrin.—The consolidated areas contain fibrin, which is very sharply picked out in sections stained by Kochel's method. In the older and more necrotic areas of consolidation the amount of fibrin is variable; in some of these situations it has almost disappeared, in others it is abundant. The areas of commencing consolidation are characterised by a dense network of delicate fibrin fibrils. Early fibrin is also found in the blood-vessels.

Minute Characters of Cells.—The chief point of interest is the large number of cells, derived from the alveolar epithelium, which have reverted to the embryonic type and commenced proliferation. Patches of this type of growth are numerous on the outskirts of the consolidated areas. The tissue is not sufficiently well preserved for minute cell differentiation, but there is clear indication of a general infiltration with multinuclear leucocytes.

Distribution of Bacilli.—Tubercle bacilli are found in moderate numbers in the consolidated areas, and are also contained within desquamated epithelial cells. The distribution of bacilli bears no noticeable relation to the deposits of fibrin.

SUMMARY.

The lungs are to a large extent consolidated. The lesions, though containing tubercle bacilli, are not, on the whole, typical of tuberculosis.

VIRUS.—H 10. B.S.

Animal Fed.—Monkey (Rhesus) 53.

Monkey 53 was fed with ·1 mg. of culture isolated from Heifer 231 and died 151 days afterwards.

Lungs.

Histological Changes.—The tissue examined is in a condition of nearly complete consolidation, a few patent alveoli being left here and there. The consolidated areas are partially caseous and, except in these situations, are densely packed with cells, which are chiefly endothelial cells, fibroblasts, and lymphocytes, multinuclear leucocytes being relatively rare; there are very few giant cells. No fibrin is present.

Distribution of Bacilli.—Bacilli are present but rare.

Liver.

Histological Changes.—Tubercles are distributed at close intervals throughout the tissue. Some of these are fairly large and centrally caseous, but the majority are

much smaller and have not commenced to caseate. Most of the tubercles contain several giant cells. The tubercles are irregular in contour, are not surrounded by a fibrous zone, and appear to be extending. Small lymphocytes are abundant ; multinuclear leucocytes are much less frequent. No fibrin is present.

Distribution of Bacilli.—Bacilli are present in most of the nodules but are not numerous ; they are often within giant cells.

Bronchial Gland.

The gland examined is entirely necrotic and contains bacilli in small numbers.

SUMMARY.

Advanced, progressive tuberculosis. Bacilli are not numerous.

VIRUS.—H 10. B.S.

Animal Fed.—Monkey (Rhesus) 55.

Monkey 55 was fed with 1 mg. of culture isolated from Heifer 231, and was killed 99 days afterwards.

Lungs.

Histological Changes.—The tissue is almost completely consolidated and resembles the lung of Monkey 53, but multinuclear leucocytes are more plentiful. Early fibrin is abundant and stains well.

Distribution of Bacilli.—Bacilli are scanty.

Liver.

Histological Changes.—The lesions resemble those of Monkey 53 and consist of miliary tubercles closely packed together ; the larger of them are caseous.

Distribution of Bacilli.—Bacilli are present in the lesions, and, though not abundant, are more numerous than in the liver of Monkey 53.

Kidneys.

In the specimen examined there is a caseous focus of considerable size in the cortex. It contains giant cells and has no definite boundary, but merges into the surrounding interstitial tissue, which is infiltrated with plasma cells and small lymphocytes. Bacilli are present in the nodule, but are rare.

Hepatic Gland.

Histological Changes.—Very advanced caseation.

Distribution and Characters of Bacilli.—Bacilli are very abundant. They vary in length from 2 to 4μ, the longer forms being frequently curved and beaded.

SUMMARY.

The disease is similar to that produced in Monkey 53.

VIRUS.—H 10. B.S.

Animal Fed.—Monkey (Rhesus) 59.

Monkey 59 was fed with 10 mg. of culture isolated from Heifer 231, and was killed 79 days afterwards.

Lungs.

Histological Changes.—The tissue is in a semi-solid condition and consists of irregular, confluent, caseating miliary tubercles, between which are the remains of more or less disintegrated alveoli.

Distribution of Bacilli.—Bacilli are very abundant in all the consolidated tissue and are also found in considerable numbers in and amongst the epithelium of patent alveoli.

Liver.

Histological Changes.—The tissue is packed throughout with innumerable minute tubercles. These are very closely crowded together and in many places no more than a network of intact liver cells is left between them. The largest of these tubercles are partially necrotic and are filled with broken down nuclear material, leucocytes and small lymphocytes. In tubercles of a somewhat smaller size there is no nuclear débris, small lymphocytes and plasma cells are frequent, while multinuclear leucocytes are present in much smaller numbers ; only shadows of the liver cells are left, and there is a marked increase in the endothelial element. In the smallest tubercles, many of which are not more than 5 or 6 cells in diameter, the liver cells are clearly recognisable, though their nuclei have lost their regular shape ; the endothelial cells are conspicuous and have swollen nuclei, but are not greatly increased in number ; the only other cells present are occasional lymphocytes and leucocytes, the former being more frequent than the latter. Close to one of those minute tubercles a liver cell has been seen in mitotic division.

Distribution of Bacilli.—Bacilli are present in all the tubercles. Even in the smallest several bacilli are to be found, and in the largest and oldest tubercles the number of bacilli is high. In the smallest tubercles and in situations which have not yet become tubercles bacilli are frequently present within liver cells.

Kidneys.

Histological Changes.—Small, irregularly-shaped tubercles, in various stages of caseation and apparently extending along the interstitial tissue, are of frequent occurrence. Throughout the section, but more markedly in the neighbourhood of the tubercles, the interstitial tissue is increased by an infiltration which consists mainly of small lymphocytes, though plasma cells and multinuclear leucocytes are also present in it.

Distribution of Bacilli.—Bacilli are present in the caseous areas and in some of the more advanced are very abundant. They have not been noted in the interstitial infiltration apart from these areas.

Thoracic Gland.

The gland examined is extensively caseous and contains bacilli in moderate numbers.

Mesocolic Gland.

The gland examined is extensively caseous and contains fairly large numbers of bacilli.

SUMMARY.

Advanced progressive tuberculosis. The number of bacilli present in the tissues is large ; in this respect Monkey 59 differs from the monkeys which were fed with smaller doses of the same material.

VIRUS—H 25. A.T.

Animal Inoculated.—Chimpanzee 1.

Chimpanzee 1 was inoculated subcutaneously with 1 mg. of culture of H 25 A.T., and died 50 days afterwards.

Lungs.

Histological Changes.—The walls of the alveoli are greatly thickened and are infiltrated with large numbers of leucocytes and lymphocytes. Here and there tubercles in an early stage of caseation are present. These tubercles are not sharply defined at their periphery, but merge into the surrounding tissue, which consists of infiltrated alveolar walls and partially occluded alveoli. The tubercles contain a deposit of fibrin.

Distribution and Characters of Bacilli.—Bacilli are numerous within the tubercles, but are not found within the thickened alveolar walls, nor in other situations not definitely tubercular. The bacilli vary in length from 2 to 4·5μ, the average being about 3·5μ. Curved forms are numerous.

Liver.

Tubercles are distributed in very large numbers. The largest of them are almost completely caseous, and the smallest are commencing to caseate. The tubercles are obviously progressive and are not bounded by a cellular zone. A few giant cells are present. Many of the tubercles show a well-marked deposit of fibrin. They contain numerous bacilli.

Kidneys.

Beneath the capsule there are small caseous patches. These have no definite boundary, but the interstitial tissue adjacent to them is densely infiltrated with lymphocytes and leucocytes. Bacilli are present in small numbers in the caseous areas.

Portal Gland.

The gland is in a condition of advanced caseation, contains large numbers of bacilli, and shows remains of a fibrinous deposit.

Spleen.

The spleen contains many large areas of advanced caseation. A few giant cells are present. Tuberele bacilli are moderately numerous in the caseous areas; they also occur in other parts of the tissue, apart from histological tubercles. The bacilli have a tendency to be long and curved.

Intestine.

In an ulcerated patch present in the small intestine no tubercle bacilli have been found.

SUMMARY.

Severe, progressive infection.

VIRUS.—H 25. A.T.

Animal Fed.—Chimpanzee 3.

Chimpanzee 3 was fed with 1 mg. of culture of H 25 A.T., and died 77 days afterwards.

Lungs.

Histological Changes.—The tissue is in a condition of patchy consolidation typical of tubercular pneumonia. All except the smallest consolidated areas are centrally caseous. There are some very large foci which are completely necrotic; these exhibit a small amount of fibrous tissue at their periphery. But the majority of the foci have no definite boundary; they are partially coalescent, and ramify into the adjacent alveolar walls, which are greatly thickened. The tubercles and the tissue adjacent to them are densely infiltrated with multinuclear leucocytes. A few giant cells are present. There is an abundant deposit of fibrin.

Distribution and Characters of Bacilli.—Bacilli are present everywhere, and are particularly abundant in the older caseous foci. Even where there is some evidence of an outer fibrous zone, bacilli are present in large numbers both in this region and immediately external to it. Their length varies from about 1·5 to 4μ. Curved and straight forms are about equally numerous.

Liver.

The liver is in a condition of fatty degeneration. Small miliary tubercles are thickly distributed throughout its substance. These tubercles are only slightly caseous and have no definite boundary wall. They contain numerous giant cells, are infiltrated with multinuclear leucocytes, and show an increase of cells which are probably of endothelial origin. Bacilli are present in the tubercles, but are not numerous. There are some small deposits of fibrin.

Kidneys.

Many small early lesions are present. Some of them involve, and appear to originate from, glomeruli. Bacilli are present in all these foci, but not in large numbers; they do not form groups. No fibrin is present.

Bronchial Glands.

The gland examined is completely caseous throughout, and contains very large numbers of bacilli.

Spleen.

The spleen is permeated with irregular, slightly caseous areas, and contains a great many giant cells. Bacilli, though found in all the lesions without difficulty, are not numerous. Most of the giant cells contain a bacillus.

Intestine.

An ulcer present in the small intestine contains numerous bacilli.

SUMMARY.

Generalised tuberculosis, with particularly severe incidence on the lungs.

VIRUS.—H 28. C.L.

Animal Fed.—Monkey (Rhesus) 63.

Monkey 63 (young) was fed once with the infected milk of Cow 143, the dose being estimated to contain 5 million bacilli. The monkey died 88 days afterwards.

Lungs.

The lung is in a condition of pneumonia, with intervening tracts of air containing tissue. The consolidated areas are crowded with leucocytes and lymphocytes, and in some situations are just commencing to necrose. Tubercle bacilli are found in all parts of the tissue, and in the consolidated areas are extremely abundant, forming dense clusters and colonies.

Liver.

The tissue is riddled with small miliary tubercles in an early stage of caseation. There are also some larger

tubercles in a more advanced stage of caseation. Tubercle bacilli are abundant everywhere, and in the larger tubercles form dense clusters similar to those in the lungs.

Kidneys.

Beneath the capsule there is a caseous tubercle packed with bacilli, and a smaller tubercle is present in another part of the cortex. The rest of the section contains no lesions or tubercle bacilli.

Bronchial Gland.

The gland is completely necrotic and crowded with bacilli.

SUMMARY.

Highly acute infection with advanced tissue destruction and enormous multiplication of bacilli.

VIRUS.—H 28. C.L.

Animal Fed.—Monkey (Rhesus) 65.

Monkey 65 (young) was fed once with the infected milk of Cow 143 (dose 5 million bacilli) and died 80 days afterwards.

Lungs.

Histological Changes.—The tissue is in a condition of incomplete consolidation, about half the air-spaces being obliterated. The lesions are typical of the acute tubercular pneumonic type, and consist of irregular caseous or partially caseous patches which are in various stages of development and ramify in all directions amongst groups of patent, but more or less congested, alveoli. No fibrin is demonstrable.

Distribution and Characters of Bacilli.—Bacilli are abundant in all situations, including the alveolar capillaries. Some are to be found within the endothelial cells of arteries. In many of the caseous areas they are growing into colonies and form conspicuous objects under a low power. They vary in length from 1 to 2·5 or 3μ and are for the most part straight and uniformly stained.

Kidneys.

Histological Changes.—There is a diffuse, general infiltration which consists mainly of small lymphocytes, though both fibroblasts and multinuclear leucocytes are also present in the interstitial tissue in abnormally large numbers. In several situations, particularly in the cortex, the normal renal tissue is replaced by tubercular foci of the " small-celled " type, the cells in these foci consisting chiefly of multinuclear leucocytes and a rather smaller number of lymphocytes. These foci are not separated by any boundary zone from the surrounding tissue; they do not appear, judging from their situation, to originate from glomeruli; glomeruli, when involved, appear to be involved by an extension of the original focus. No fibrin is demonstrable.

Distribution of Bacilli.—The tubercular foci referred to are packed with enormous numbers of bacilli. Bacilli are also plentiful throughout every part of the tissue, both in the interstitial infiltration and contained within renal cells. A few of the glomerular capillaries contain solid clumps of them, but, in spite of the presence of these clumps, show remarkably little tissue reaction; in sections not stained for bacilli it would be impossible to distinguish these glomeruli from glomeruli which contain no bacilli. It is evident that the tissue as a whole has lost its power of reacting against the bacilli and has been overrun by them.

Mesenteric Glands.

The gland examined contains many large caseous patches and is overrun by bacilli, which in some situations have multiplied into conspicuous colonies.

SUMMARY.

Acute, progressive tuberculosis. Bacilli very numerous.

VIRUS.—H 56. F.T.

Animal Fed.—Baboon 1.

Baboon 1 was fed with 1 mg. of culture of H 56. F.T.; and was killed, when moribund, 108 days afterwards.

Lungs.

The tissue contains large, irregularly distributed patches of consolidation, which are partially caseous and contain many giant cells. Bacilli are found without difficulty in all the consolidated areas, but generally occur singly, and nowhere form groups.

Liver.

The specimen contains large, ramifying areas of completely caseous material. At the periphery of these areas giant cells are numerous, but there is no definite boundary zone. Bacilli are rare.

Kidneys.

In the specimen examined there is a small caseous tubercle, containing very few bacilli, situated beneath the capsule.

Thoracic Gland.

The gland is almost completely caseous. Bacilli are rare.

SUMMARY.

Progressive tuberculosis of the lungs and liver; caseation advanced; bacilli scanty.

VIRUS.—H 56. F.T.

Animal Inoculated.—Baboon 3.

Baboon 3 received a subcutaneous inoculation of 1 mg. of culture of H 56. F.T. and died 38 days afterwards. Dr. Cobbett tells me that the animal did not appear in good health at the commencement of the experiment.

Lungs.

The alveolar walls are greatly thickened and are infiltrated with lymphocytes and leucocytes, but in the specimen examined only one tubercular lesion is present.

It is a small, slightly necrotic patch situated beneath the pleura and contains about half-a-dozen bacilli. No bacilli have been found in the rest of the tissue.

Liver.

Several suspicious foci have been found, but no tubercle bacilli.

Kidney.

No lesions or bacilli have been found.

Mesenteric Gland.

Large caseous areas are present. They contain a few tubercle bacilli.

SUMMARY.

Slight tuberculosis of the lungs. Advanced lesions in a mesenteric gland.

VIRUS.—H 61. E.C.

Animal Inoculated.—Monkey (Rhesus) 73.

Monkey 73 was inoculated subcutaneously with ·01 mg. of culture of H 61. E.C. (brain), and died 89 days afterwards.

Lungs.

The tissue examined is almost completely solidified, and is to a large extent caseous. No fibrin is present. Tubercle bacilli occur in enormous numbers and have grown into groups and colonies visible under a low power in nearly all parts of the specimen. They are from about 1 to 3μ in length, frequently curved, but generally stained uniformly.

Liver.

The tissue contains large, completely caseous tubercles, and also minute tubercles which are extremely numerous and are only slightly caseous. The tubercles are not bounded by any peripheral zone. No fibrin is present. In the larger tubercles bacilli are aggregated in dense masses, as in the lungs. In the smaller tubercles bacilli are much less numerous. Apart from the tubercular areas, bacilli occur everywhere, both within blood-vessels and within parenchymatous liver cells.

Kidneys.

Miliary tubercles, commencing to caseate, are abundant. Bacilli are extremely numerous and in many places have grown into dense colonies.

Axillary Gland.

Completely caseous and swarming with bacilli.

SUMMARY.

Severe, generalised tuberculosis, with free dissemination of bacilli by the blood-stream. Bacilli extremely numerous. No fibrin found.

VIRUS.—H 61. E.C.

Animal Inoculated.—Monkey (Rhesus) 75.

Monkey 75 was inoculated subcutaneously with ·1 mg. of culture of H 61. E.C. (brain) and died 84 days afterwards.

Lungs.

The tissue is congested and contains a circumscribed tubercle which is almost entirely caseous. No fibrin is present. Within the tubercle dense colonies of tubercle bacilli are present. A few bacilli are also present in other parts of the tissue.

Liver.

Identical with the liver of Monkey 73. No fibrin present.

Kidneys.

Caseous foci, swarming with bacilli, are present.

Mesenteric Gland.

The gland is very extensively caseous; no fibrin is present. Bacilli are extremely abundant and have grown into numerous colonies.

SUMMARY.

Generalised tuberculosis; extensive caseation; enormous numbers of bacilli; no fibrin.

C. SUMMARY.

These investigations provide histological evidence in support of the following results:—

SUSCEPTIBILITY.

Anthropoid apes and monkeys are highly susceptible to tuberculosis. This has been shown to be true for every species, including the chimpanzee, upon which an adequate number of experiments has been made. The lesions produced, though varying in severity in different cases, are all typical of tuberculosis.

THE PATHOGENICITY OF DIFFERENT VIRUSES.

Experiments have been made (1) with bovine viruses which are of high virulence to the bovine and the rabbit; (2) with human viruses, which are of equally high virulence to these two classes of animals, and (3) with human viruses of much lower virulence. But it has been found, and my histological evidence confirms this result, that anthropoid apes and monkeys are so highly susceptible to the disease that the viruses last mentioned, as well as the two former groups, are capable of producing in these animals severe infection.

THE MODES OF INFECTION.

When the bacilli are introduced by feeding, the results are variable. Dissemination generally occurs and lesions are usually present in the lungs; in fact, the incidence of the disease is often particularly severe upon these organs. But the lesions found in these and other distant organs differ very considerably in respect both of their numbers and their histological characters. Sometimes they are scanty and not obviously progressive; at other times they are more numerous and, though of the "chronic" type, exhibit more or less decisive evidence of progression; and at other times again a distinctly acute type of tuberculosis has been produced.

My histological examinations afford evidence that subcutaneous inoculation is a more certain method of producing severe disease, and they also demonstrate the extreme severity of the type of disease which is produced by intravenous inoculation.

EXPERIMENTS ON GOATS.

A.—LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B II.

Animal Inoculated.—Goat 10.

Goat 10 was killed 165 days after subcutaneous inoculation with emulsion from Calf 134 containing 4,480,000 bacilli.

Lungs.

Histological Changes.—The lungs were extensively caseous and contained numerous cavities. The portion of tissue selected for examination was one where the lesions had not advanced to cavity formation. The section exhibits to the naked eye discrete nodules from 2 to 4 mm. in diameter. On microscopic examination these are found to be composed of a fusion of smaller tubercles. In some of these tubercles the process of caseation is very advanced, in others it is just commencing, and intermediate stages are seen. The individual tubercles have a broad but loosely constructed cellular and fibrous zone which blends with the environment of adjacent tubercles, but leaves here and there small patches of incompletely solidified tissue where remains of alveoli can be seen. This tissue which blends the tubercles together is in contrast with the tougher fibrous tissue strands which constitute the normal septa passing into the lung and which are not broken down by the advance of the tubercular process. The tubercular areas contain many giant cells. Apart from these aggregations of tubercles the lung substance shows little that is abnormal, with the exception of patches of partial collapse and patches of slight emphysematous distension of the alveoli.

There are masses of oxyphil leucocytes in the tubercles which are commencing to caseate, and, in the older foci, at the outer margin of the caseous area; but in other situations these cells are scanty. The cellular material which surrounds the individual tubercles and binds the tubercles together consists mainly of large flattened cells which, when cut at right angles to their flat surface, exhibit a long, narrow nucleus with a long line of protoplasm continued beyond either extremity; when cut obliquely or when the section passes parallel to their flat surface, their nuclei appear oval and their protoplasm is broader, imperfectly defined, and often runs out into linear processes in various directions. These cells exhibit a marked tendency to fuse together. They are also frequently found running in parallel columns; between these columns red blood corpuscles can sometimes be seen. The cells referred to can in some situations be traced to the capillary endothelium of alveoli from which the epithelial cells have desquamated, and it appears clear that they are of endothelial origin. Plasma cells are numerous, especially at the periphery of the tubercles. Both the rounded forms and the forms passing into fibroblasts are present.

Distribution of Bacilli.—Bacilli are present, but extremely rare.

Liver.

The liver contains circumscribed tubercles which are in an advanced stage of caseation and are surrounded by a dense fibrous wall. No bacilli have been found in the specimens examined.

Mesenteric Gland.

The gland examined is extensively caseous and contains tubercle bacilli in small numbers.

SUMMARY.

Disseminated tuberculosis with extensive caseation. Tubercle bacilli rare.

VIRUS.—B IV.

Animal Fed.—Goat 20.

Goat 20 (young) was killed 131 days after being fed with 10,150,000 bacilli contained in the milk of Cow 164.

Lungs.

The specimen contains numerous tubercles which are in various stages of caseation. In the oldest lesions the caseation is advanced and at their periphery there is an irregular distribution of fibroblasts. The earlier lesions, where caseation is commencing, show no evidence of being surrounded by fibroblasts. Many of the tubercles are confluent, and it is noticeable that external to the periphery of the larger tubercles there are aggregations of smaller tubercles in process of formation. It is evident, therefore, that the lesions are progressive. The lesions are densely invaded with finely granular oxyphil leucocytes, amongst which mononuclear forms are numerous. Plasma cells are also abundant. Tubercle bacilli are very numerous and have grown everywhere into small clusters. They are not confined to the definitely tubercular foci, but have evidently passed from these situations into the surrounding tissue.

Liver.

Histological Changes.—Small tubercles, generally not larger than half the diameter of the microscopic field (low power), occur with moderate frequency throughout the liver. Frequently they are distinctly intralobular; sometimes they involve both the interlobular interstitial tissue and the adjacent parenchymatous cells. They often contain one or two giant cells. With the exception of these miliary tubercles there are no histological lesions noticeable with a low power. There is an absence of interstitial infiltration.

Throughout the liver the blood capillaries contain an abnormally large number of multinuclear leucocytes. The structure of the tubercles is as follows. There is a framework, which consists partly of parenchymatous liver cells and partly of endothelial cells. The liver cells are in various stages of disintegration. Their protoplasm tends to disappear before their nuclei, but the process of disappearance is not abrupt. The protoplasm belonging to different cells fuses together, so that the cell outlines are lost. As the process goes on, the protoplasm which survives assumes the appearance of irregular branchings and filaments, thus giving rise to the so-called tubercular reticulum. The nuclei of the liver cells, though surviving longer, soon lose their circular contour and assume a variety of outlines. The endothelial cells show a greater vitality than the liver cells, and multiply. Their development appears to be determined by an effort to cover with a lining membrane the irregular gaps and clefts produced by the morbid process which is going on in the liver parenchyma. As these gaps and clefts are highly irregular, the development of the endothelial cells is also one of great irregularity. It leads to many varieties in their nuclear outlines and also to the formation of giant cells. In eosin and methylene blue specimens the nuclei of these giant cells often show minute red nucleoli. Turning to the normal cells, these red nucleoli are only present in the parenchymatous liver cells and the endothelial cells of the capillaries. The evidence of the greater activity of the endothelial cells as compared with the liver cells is so strong that the possibility of the giant cells being due to multiplication of liver cells may reasonably be excluded. Possibly prolongations of endothelial cells assist, to some extent, in the formation of the reticular appearance.

The only other cells present in the tubercles are leucocytes and small lymphocytes. The leucocytes are very numerous and are all of the finely granular oxyphil type, though when found in a condition of partial disintegration their protoplasm does not stain. They are not all multinuclear; several have a small, deeply staining spherical nucleus surrounded with a halo of fine eosinophil granules. The lymphocytes are few in number, in proportion to the leucocytes. No fibrin is present.

Distribution of Bacilli.—Bacilli are rare. Occasionally one or two are to be found in a tubercle.

Lymphatic Glands.

A small cervical gland examined contains large breaking-down areas, and is everywhere swarming with bacilli. The bacilli have multiplied into minute colonies, which appear under a low power as red points thickly scattered all over the section. The bacilli are generally from 2 to 3μ in length, but sometimes rather longer. The longer forms are often slightly curved. There is some tendency to beading, but discretely beaded forms are rare. The gland contains dense masses of oxyphil leucocytes.

A mediastinal gland which was examined proved to be in a similar condition, but rather less advanced.

SUMMARY.

Disseminated tuberculosis; notably progressive in the lungs. Bacilli abundant in the glands draining the alimentary tract and in the lungs, but scanty in the liver.

VIRUS.—B V.

Animal Inoculated.—Goat 2.

Goat 2 (adult) died 54 days after subcutaneous inoculation with an emulsion of original material of B V. containing 4,500,000 bacilli.

Lungs.

Histological Changes.—On post-mortem examination a considerable portion of the lung tissue was found to be honeycombed with small cavities. Less advanced lesions were selected for histological purposes.

The tissue examined is found to be beset with numerous small tubercles in an advanced stage of caseation. The tubercles are variable in size, irregular in outline, and often confluent. They are evidently increasing in area, and the alveoli adjacent to them show a desquamation of epithelium and a cellular infiltration. In the rest of the lung tissue there is a well-marked compensatory emphysema. Large giant cells are numerous both within and adjacent to the tubercles.

The tissue is somewhat necrotic and the cell details are not very clearly brought out, but the following features are observable. At the margin of the tubercles there is a fairly extensive zone, in the case of the older lesions, of fibrous tissue; but the fibroblasts are breaking down and becoming overrun with leucocytes, both multinuclear and mononuclear, the former being always abundant and often in excess of the latter. In the partially consolidated areas immediately external to the tubercles multinuclear leucocytes are again very abundant, and together with desquamated epithelial cells, lymphocytes, and swollen capillary endothelial cells fill up the alveoli. In van Gieson specimens some increase of adult fibrous tissue is shown.

No fibrin is demonstrable.

Distribution and Characters of Bacilli.—Within some of the caseous nodules the bacilli have grown into dense colonies, but with these exceptions bacilli are relatively infrequent. Some of the tubercular nodules contain few or none, and there are many giant cells in which none can be seen. The bacilli are evidently unable to multiply freely in the tissue except in certain specially favourable situations.

The bacilli are from about 2 to 5 μ in length. The greater number of them are curved, and beaded forms are present, though not very common.

Right Prescapular Gland.

The animal was inoculated on the left side of the neck.

Histological Changes.—Extensive areas of complete necrosis; numerous smaller areas of partial necrosis.

The only feature of interest is the presence of an exceptionally large number of giant cells at the periphery of the necrotic areas and amongst the less advanced lesions. These cells are often 60μ or more in diameter.

Distribution of Bacilli.—Bacilli occur, but rather scantily, in all the necrotic areas and within most of the giant cells.

SUMMARY.

Advanced, progressive, caseous tuberculosis of the lungs. In some of the caseous foci there are dense masses of tubercle bacilli, but in most of the lesions bacilli are scanty.

VIRUS.—B V.

Animal Inoculated.—Goat 28.

Goat 28 died 91 days after subcutaneous inoculation with ·01 mg. of culture from G.P. 711, which had been inoculated with original material.

Lungs.

Histological Changes.—The specimen examined shows a large caseous nodule surrounded by a broad, circular, cellular zone. The rest of the lung tissue is not involved in the tubercular process and shows no important pathological change.

The framework of the cells circumscribing the tubercle is of the endothelial type, the individual cells and their general arrangement corresponding in every detail to the description given in reference to the lung of Goat 10. This cellular framework is swarming with oxyphil leucocytes; it also contains a very broad zone of plasma cells, which occur side by side with large numbers of multinuclear leucocytes, but do not penetrate so far into the interior of the nodule as the latter. The plasma cells exhibit every variety of form, from the large circular cell to the elongated spindle-shaped cell. They stain deeply and are sharply distinguishable from the endothelial type of cells referred to above, which take up little or no pyronin stain.

Distribution of Bacilli.—Very few bacilli are to be found.

Liver.

Histological Changes.—The specimen examined contains a large tubercular nodule which on closer examination proves to consist of a fusion of smaller nodules. The general structure of the lesion closely corresponds to the lesion described in the lung of Goat 10.

The cells of the endothelial type are well shown and can be traced into the liver substance external to the tubercle. Oxyphil leucocytes are numerous, but not so numerous as in the lung of this animal; some of those at the periphery of the nodule are of the coarsely granular type. Small lymphocytes are also plentiful. There is a well-marked zone of plasma cells.

Distribution of Bacilli.—Bacilli are present in the nodule, but are very rare.

Bronchial Gland.

A bronchial gland examined exhibited advanced caseous lesions, with very few bacilli.

SUMMARY.

Disseminated, caseous tubercles containing few bacilli.

B.—LESIONS PRODUCED BY HUMAN BACILLI.

VIRUSES.—H 8. S.C., H 32. Y.W.

Animal Inoculated.—Goat 19.

Goat 19 was inoculated subcutaneously on three successive occasions. First, with emulsion from Calf 305 (H 8. S.C.) containing 12,550,000 bacilli; 135 days subsequently, with emulsion from Calf 361 (H 8. S.C.) containing 3,000,000 bacilli; 146 days subsequently, with emulsion from Calf 487 (H 32. Y.W.) containing 310,300,000 bacilli. Goat 19 died 35 days after the last inoculation.

Lungs.

Histological Changes.—The lung is in a condition of nearly complete consolidation. In the specimen examined, four small groups of alveoli are found, which are empty and distended, whilst the rest of the tissue is deeply congested, and consists, in about equal proportions, of completely consolidated tissue where the alveoli are obliterated and tissue where the alveoli are more or less filled with cellular material. The completely consolidated tissue consists of irregular, extensive, partially necrotic tracts which merge without any line of demarcation into the surrounding partially consolidated areas. There are no conspicuous giant cells.

The tissue contains very large numbers of multinuclear leucocytes which are distributed everywhere. There is some little evidence of an attempt at fibrous tissue formation in the less necrotic parts of the consolidated tissue. Plasma cells occur in rings round the blood-vessels and bronchioles, and are distributed scantily throughout the rest of the tissue. Large lymphocytes are rather frequent in the blood-vessels. The alveoli are filled with desquamated epithelial cells and numerous multinuclear leucocytes. The tissue was probably slightly necrotic as it does not react well to delicate stains. There is some deposit which takes a fibrinous stain, but no distinctive fibrin.

Distribution and Characters of Bacilli.—Bacilli are scattered throughout the tissue in large numbers, but are not sufficiently abundant to be readily recognisable with a low power. They have a tendency to form little groups, but only very small groups; it is unusual to find more than a dozen bacilli in actual contact with each other. A large proportion of the bacilli are intracellular; they are particularly common within epithelial cells, where they have generally succeeded in growing, and formed the little groups referred to above. They are also frequent within endothelial cells and fibroblasts. Occasionally they are seen within a multinuclear leucocyte; possibly the majority of the bacilli, which were readily accessible to the attacks of the leucocytes, have already perished. None are found within the blood-vessels.

The bacilli average about 3μ in length, are slightly curved, and have a slight tendency to beading.

Thoracic Gland.

A thoracic gland contains many minute early foci which are only slightly caseous. In these foci there are small groups of bacilli.

SUMMARY.

The lung is in a condition of nearly complete consolidation and is extensively caseous. Tubercle bacilli are numerous.

VIRUS.—H 10. B.S.

Animal Inoculated.—Goat 3.

Goat 3 was killed 47 days after subcutaneous inoculation with emulsion from Calf 113 containing 1,800,000 bacilli.

Lungs.

Histological Changes.—The lungs are in a condition of incomplete consolidation. Where the process is most advanced only a few of the air spaces are left unobliterated; in situations less extensively affected the consolidated and the air-containing tissue are about equal in amount and there is some compensatory emphysema. Distributed amongst the consolidated tissue are numerous areas of caseation; many of these are circular in outline and discrete, others are partially confluent. No giant cells are seen. The tissue surrounding the caseous areas consist of a confused mass of breaking down cells, of which many are of the type of young fibroblasts and many are multinuclear leucocytes. The general characters of the lesions are those of a progressive necrosis which is being resisted, though unsuccessfully, by an attempt at fibrous tissue transformation. There is well marked destruction of the elastic fibres. No fibrin is present.

Distribution of Bacilli.—Bacilli are very scanty.

SUMMARY.

The lungs are in an advanced condition of tubercular pneumonia. Bacilli are scanty.

VIRUS.—H 14. F.S.

Animal Inoculated.—Goat 5.

Goat 5 died 75 days after subcutaneous inoculation with emulsion from Heifer 197 containing 4,500,000 bacilli.

Lungs.

Histological Changes.—The lung contains large tubercles in an advanced stage of caseation. External to these, the alveoli are occupied by desquamated epithelium and a cellular infiltration ; and, passing further outwards, there is an infiltration of the alveolar walls. These processes are evidently radiating outwards from the tubercles. Some areas of the lung tissue are normal, but, in the specimens examined, the tubercles are so near together that these areas are relatively small. Giant cells are found within the periphery of the tubercles. The tubercles are surrounded by a well-defined area of plasma cells, and cells of this type are also found in the adjacent alveoli and alveolar walls where the epithelium has desquamated. There are numerous aggregations of multinuclear leucocytes.

Distribution of Bacilli.—Bacilli are scanty and are only found within the nodules ; they generally occur within partially broken down cells which it is impossible to identify with certainty.

Thoracic Gland.

Histological Changes.—The gland is extensively necrotic.

Distribution of Bacilli.—The bacilli are scanty, but are to be found in most of the lesions, including many of the giant cells. No bacilli occur in the lymph sinuses, either intracellular or free.

SUMMARY.

Progressive caseous tuberculosis of the lungs. Bacilli scanty.

VIRUSES.—H 17. Sp. B., H 28. C.L., H 32. Y.W.

Animal Inoculated.—Goat 1.

Goat 1 was inoculated subcutaneously on four successive occasions ; (1) with an emulsion from Calf 161 (H 17. Sp. B.), containing 33,330 bacilli ; (2) 253 days later with an emulsion from guinea-pigs of Calf 339 (H 17. Sp. B.), containing 31,272,000 bacilli ; (3) 35 days later with an emulsion from Calf 515, (H 28. C. L.) containing 10,000,000 bacilli ; (4) 72 days later, with an emulsion from Calf 487 (H 32, Y. W.), containing 620,600,000 bacilli. The goat died 34 days after the last inoculation.

Lungs.

Histological Changes.—The entire section shows advanced congestion, and most of the alveoli are completely or partially filled with desquamated epithelium and other cellular material. Miliary tubercles are numerous everywhere. They are centrally caseous, semi-confluent, and not sharply marked off from the rest of the tissue. There are no giant cells.

The cells are too much affected by the general œdematous condition to stain well. The tubercular areas, where not too necrotic, show a confused mass of broken-down leucocytes, lymphocytes and fibroblasts. The general appearance suggests that a conservative process of fibrous tissue formation had previously been at work, but has recently been overcome, Plasma cells, though staining imperfectly, can be definitely recognised in all parts of the tissue ; they are most conspicuous at the periphery of vessels and bronchioles. The whole tissue is invaded with multinuclear leucocytes. The alveoli and bronchioles are crowded with these cells, entangled with desquamated epithelial cells. Some of the leucocytes retain their oxyphil stain, an indication that they are still in an active condition. There is considerable destruction of elastic fibres.

Very little fibrin is present, but a few delicate strands can be found, sometimes in the vessels and sometimes amongst tissue cells.

Distribution and Characters of Bacilli.—The tissue is overrun with bacilli, which appear under a low power as a sprinkling of fine specks, visible everywhere and distributed fairly equally. It is noteworthy that though abudant in the tubercles they are not more numerous there than in other situations. Alveoli and bronchioles everywhere are crowded with them. In these situations they are contained within alveolar cells, and, very frequently, within leucocytes. The bacilli have a tendancy not so pronounced as in the rat, but still unmistakable, to grow in a continuous mass round cell nuclei. The general impression is that though the bacilli have eventually gained the upper hand, the animal has offered a vigorous resistance. The presence of large numbers of bacilli where there is no necrotic change, the capacity of cell nuclei to retain their staining properties although surrounded with bacilli, and the fact that there is still a vigorous resistance on the part of the multinuclear leucocytes afford substantial evidence in support of this view.

The bacilli are from 2 to 4·5 μ in length and the longer forms are more frequently curved than straight. Beaded forms occur, but are relatively infrequent.

Thoracic Gland.

Histological Changes.—Blood vessels intensely engorged. Many small pale areas in the cortex. Multinuclear leucocytes, many of which have lost their protoplasmic staining properties, are numerous in these areas and are still more numerous in the lymph channel immediately beneath the capsule. Some patches of fairly recent fibrin are present and are associated with bacilli.

Distribution and Characters of Bacilli.—In all the pale areas referred to bacilli are abundant. They are still more abundant all along the lymph space beneath the capsule. In both these situations they are evidently being vigorously attacked by the multinuclear leucocytes. Some of these cells contain bacilli within their protoplasm, and there are many broken down leucocytes in close association with groups of bacilli. Bacilli are very frequently found within the large mononuclear cells which occur in large numbers lying free within the peripheral lymph sinus. These cells are often vacuolated and sometimes contain within their protoplasm one or two nuclei of cells which they have absorbed. Bacilli are also found attached to or within endothelial cells and fibroblasts. In the substance of the gland, and apart from the regions which are histologically tubercular, isolated bacilli are often present. In this gland therefore, as in the lung, there is abundant evidence of phagocytic action. At the same time bacilli are also found which have evidently succeeded in growing within cells, and are tending to surround the cell nuclei. The parasitic action of the bacilli upon the cells must therefore be borne in mind, as well as the phagocytic action of the cells upon bacilli.

In specimens stained with methylene blue, but not with carbol-fuchsin, many of the intracellular bacilli, particularly those within large mononuclear cells, are demonstrable. These bacilli cannot, however, be regarded as having lost their acid-fast property, since in specimens stained with carbol-fuchsin and counter-stained with methylene blue, red bacilli alone are seen.

The bacilli are from 2 to 5, or occasionally 6μ in length. Straight and curved forms are about equally numerous. Beaded forms are not very common. Many of the bacilli within cells are fragmentary.

Local Lesion.

The local lesion formed at the seat of the last inoculation does not call for detailed description. It consists of the usual mass of caseous material, fragments of leucocytes and other cells ; it is bounded and intersected by broad areas of fibrous tissue ; and it is swarming with bacilli.

Two points are worth noting. (1.) In the process of tissue destruction the vascular channels survive until very late. In the sections examined, vessels filled with normal and obviously circulating corpuscles are often found in the midst of caseous areas. They are surrounded by a zone of bacilli. It is obvious that in the development of the caseous process the walls of vessels similarly situated must often have broken down, with the result that large numbers of bacilli were carried direct to the lungs.

(2.) At the periphery of the lesion, where we get early intramuscular infiltration, the process of repair by fibrous tissue formation seems to be gaining the upper hand.

SUMMARY.

Very severe infection of the lungs; tubercle bacilli present in enormous numbers.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Goat 39.

Goat 39 (young) was killed 92 days after subcutaneous inoculation with emulsion from Calf 391, containing 102,000,000 bacilli.

Lungs.

Histological Changes.—The lung contains numerous small tubercles, their area generally being not greater than that of the microscopic field (low power). The tubercles are generally well defined, and have a dense cellular zone peripherally. Interior to this are many giant cells. Some of the tubercles are completely caseous in the centre. Tubercles which are only very slightly caseous are also found. The rest of the tissue shows very considerable thickening and infiltration of the alveolar walls, with consequent compression of many of the alveoli; but there are no appearances in this tissue suggestive of the commencing formation of new tubercles. There are patches of compensatory emphysema.

Finely granular oxyphil leucocytes are very numerous throughout the tissue, and their protoplasm stains well. A point of difference from some of the more acute lesions is that in this specimen very few of the forms with completely spherical nuclei are present; they are nearly all multinuclear. Even where surrounded by completely caseous tissue, some of these cells retain their characteristic protoplasmic stain. Plasma cells form an irregularly defined layer round the tubercles and round most of the bronchi; they also occur in isolation amongst the rest of the tissue. Small lymphocytes constitute a very considerable proportion of the cellular layer forming the periphery of the tubercles, and, in the case of those which are only slightly caseous, penetrate into its interior. Most of the elastic tissue has disappeared from the tubercles. There is very little increase of adult fibrous tissue demonstrable in van Gieson specimens.

Distribution of Bacilli.—Bacilli are rare, but a few, from two to half a dozen, can be found in most of the tubercles. They very frequently occur within giant cells.

Bronchial Gland.

Histological Changes.—The bronchial gland examined shows many rather small, irregular patches of early necrotic change containing many large giant cells. Some rather larger and completely caseous nodules are also present. Oxyphil leucocytes occur, particularly in the tubercular nodules, but are not at all numerous.

Distribution of Bacilli.—Bacilli are present, but rare.

SUMMARY.

The lungs contain numerous disseminated tubercles which are not obviously progressive, though most of them contain a few bacilli.

VIRUS.—H 31. L.F.

Animal Fed.—Goat 35.

Goat 35 was fed with 10 mg. of culture of H 31 L.F., and was killed, when moribund, 106 days afterwards.

Lungs.

The lungs show many isolated caseous nodules at the periphery, of which there are dense masses of multinuclear leucocytes. In some of these nodules fairly large groups of bacilli are present.

Liver.

Isolated, caseous tubercles are present. The larger of these nodules are surrounded by fibrous tissue. Bacilli are present in the lesions in scanty numbers.

Kidneys.

No tubercles or tubercle bacilli have been found.

Thoracic Gland.

Large areas of advanced caseation are present. Bacilli are scanty.

SUMMARY.

Disseminated caseous tubercles are present in the lungs and liver.

VIRUS.—H 32. Y.W.

Animal Inoculated.—Goat 7.

Goat 7 was killed 27 days after subcutaneous inoculation with emulsion from Calf 487, containing 155,000,000 bacilli.

Lungs.

Histological Changes.—Miliary tubercles, some discrete and some confluent, are thickly scattered throughout the tissue. The tubercles have rarely advanced to definite caseation, but generally contain a central mass of nuclear fragments which retain nuclear stains. There is no sharp demarcation between the tubercles and the rest of the tissue. Surrounding the tubercles the alveoli are partially or completely filled with epithelial and other cells. External to this region the alveoli are patent, but the alveolar walls are thickened and infiltrated, the change diminishing in amount as we pass further away from the tubercle. Here and there groups of apparently normal alveoli are seen. Notwithstanding the large number of tubercles, the amount of air space remaining is very considerable. There are no conspicuous giant cells.

There is a very active leucocytosis. Finely granular oxyphil leucocytes are abundant everywhere, particularly

in the consolidated areas. Together with the detatched or disintegrated epithelial cells these leucocytes form the chief constituent of the tubercular regions which are not necrotic. These leucocytes are not all multinuclear; many of them have a perfectly spherical deeply staining nucleus, and are only distinguishable from small lymphocytes in eosin and methylene blue preparations, where, under an oil-immersion lens, their nuclei are seen to be surrounded with finely granular eosinophil protoplasm. No mitotic figures have been noted amongst the epithelial cells, but the number of these cells is so large in the consolidated areas that they must, at an earlier period have undergone multiplication. Small lymphocytes, though to be found nearly everywhere, are very few in proportion to the leucocytes. Plasma cells are also rare. The amount of endothelial proliferation or fibrous tissue formation is only slight.

Distribution and Character of Bacilli.—Bacilli are found wherever there is any consolidation, but never form large aggregations. They nearly all occur singly and are very frequently within cells, very often within epithelial cells, sometimes within endothelial cells or fibroblasts, and occasionally within multinuclear leucocytes. It seems probable that the bacilli have only been able to multiply with difficulty after arriving in the lung. Though histologically the lesions are obviously progressive, the absence of even small clusters or colonies of bacilli suggests that the resistance on the part of the tissue is very considerable. Very likely the leucocytes have already destroyed a large number of them. Those which survive apparently find the conditions of existence less unfavourable within an epithelial cell than in situations where they would be more directly exposed to the attacks of leucocytes.

The bacilli vary in length from 2 to 3 and occasionally 4μ. About two-thirds of them are straight and uniformly stained. The longer forms are often slightly curved. Beaded forms are rather exceptional.

Liver.

Small miliary tubercles occur with frequency throughout the liver. In distribution and characters they resembled those in the liver of Goat 20 (B IV), which are described in detail above.

Kidneys.

Histological Changes.—Small streaks and patches of infiltration in the cortex, sometimes involving the interstitial tissue only, but occasionally including the glomeruli as well. In the medulla, occasional streaks of infiltration, less marked than in the cortex.

In these situations there are definite but small aggregations of finely granular oxyphil leucocytes. Small lymphocytes are about equally numerous. The renal cells are very little affected. In the same situations plasma cells are numerous and stain well. They show every transitional form from the cell resembling the ordinary fibrous tissue corpuscle to the cell resembling a large lymphocyte. Apart from these situations, plasma cells occur in small numbers in the interstitial tissue where the change is so slight as not to be noticeable with a low power.

Distribution of Bacilli.—Bacilli are very rare, but a few have been found.

Thoracic Gland.

Several small areas of partial necrosis. Bacilli are confined to these situations, where they occur with moderate frequency, but generally each bacillus is isolated from the rest. There is, therefore, no evidence of recent multiplication. No giant cells have been observed. There are no features of particular histological interest.

Local Lesion.

The piece of tissue examined was found to be highly necrotic, but still vascular in parts. Bacilli were present but scarce.

Spleen.

The spleen contains many caseous patches in which tubercle bacilli occur with moderate frequency.

Mammary Gland.

In one specimen there is a small tubercle which is commencing to caseate and contains several tubercle bacilli.

SUMMARY.

Disseminated tuberculosis. The mammary gland is infected.

C. SUMMARY.

Severe, progressive disease has been produced in goats both by subcutaneous inoculation and by feeding. The lesions produced by the bovine viruses are identical in histological characters with those produced by the human viruses. All the viruses which have produced lesions of marked severity are known to be of high virulence both to the bovine and the rabbit.

Goat 39, which was inoculated from Calf 391, a strain of H 17. Sp. B., did not show acute disease. This strain, as shown by the relatively small amount of disease produced in Calf 391, was of low virulence for bovines. Other viruses or strains of low bovine virulence which have been inoculated into goats have only produced minimal lesions, and no specimens of these have been received for histological examination. Like the bovine, the goat appears to possess a high power of resistance against the bacilli of lower virulence.

EXPERIMENTS ON SWINE.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.
Animal Fed.—Pig 8.

Fed for 92 days with milk of Cow 40. Killed 247 days after commencement of experiment. Age at commencement of experiment, six weeks.

Lungs.

Histological Changes.—A few circumscribed tubercles are present. They are calcareous, have a broad fibrous tissue boundary, and contain some groups of multinuclear leucocytes.

Presence of Bacilli.—Several sections have been searched, but no tubercle bacilli have been found.

SUMMARY.

The lung contains some chronic tubercles, in which no bacilli have been found.

VIRUS.—B I.
Animal Fed.—Pig 18.

Fed, when 10 weeks old, with 1,000,000 bacilli contained in the milk of Cow 64. Killed 39 days afterwards.

Lungs.

Histological Changes.—In specimens taken from two situations there are irregular areas in which the alveolar walls are thickened with an infiltration consisting mainly of multinuclear leucocytes and small lymphocytes. In one specimen two small tubercles are present, the larger being about 1·5 mm. in diameter. These tubercles are only slightly necrotic.

Presence of Bacilli.—A few bacilli are present in these tubercles.

Mesenteric and Ileo-Colic Glands.

Histological Changes.—Both a mesenteric and two ileo-colic glands examined show advanced and generalised infection, consisting of irregular caseating areas. The necrotic change is more extensive in the ileo-colic gland. All the lesions are densely packed with multinuclear leucocytes. These cells are also found in large numbers in the surrounding areas which are not definitely tubercular. In both situations the cells are of the same type, but the granulation of the cells is much coarser in the regions external to the tubercular foci than within the tubercular foci. In the latter situation the protoplasm of the majority of the cells takes a uniform eosinophil stain with distinct granulation.

Distribution of Bacilli.—Bacilli are scanty and always occur singly. A few can be found without much difficulty in most of the caseous areas. They are less numerous in the mesenteric than in the ileo-colic glands.

SUMMARY.

Occasional small tubercles, in which a few bacilli are present, are found in the lungs. Bacilli are rare in the mesenteric and ileo-colic glands.

VIRUS.—B I.
Animal Fed.—Pig 20.

Fed, when 10 weeks old, with 10,000,000 bacilli contained in the milk of Cow 64. Killed 115 days afterwards.

Lungs.

Histological Changes.—The tissue examined contains large numbers of circular, isolated nodules, almost all in an advanced stage of caseation, and varying in diameter from a few millimetres to one-fifth the diameter of the microscopic field (low power).

All the tubercles are crowded with multinuclear leucocytes; some of the leucocytes are coarsely granular. Small lymphocytes, though not rare, are less plentiful; they are scattered about the tissue, but nowhere form definite aggregations, neither at the periphery nor in the interior of the tubercles. The framework of the tubercles consists mainly of cells which are probably due to the proliferation of endothelial cells; amongst these are the remains of desquamated epithelial cells. There is very little permanent fibrous tissue formation. In sections stained by Pappenheim's method there are no definite groups of typical plasma cells. There is not, for example, a well-marked zone of these cells at the periphery of either the tubercles, the bronchioles, or the blood-vessels. There are, however, many cells throughout the tissue which take a well-marked pyronin stain. Most of these cells are indistinguishable from large lymphocytes. They are found with moderate frequency within blood-vessels. The general appearance and distribution of these cells suggest that those present in the tissues are, at least in the majority of cases, large lymphocytes which have escaped from the blood-vessels.

Distribution of Bacilli.—Many of the nodules contain a few bacilli.

Submaxillary Lymphatic Gland.

Histological Changes.—The gland examined is in a condition of very advanced caseation. The caseous areas are still, to some extent, vascularised.

Distribution of Bacilli.—Tubercle bacilli are rather rare, and never occur in groups.

Cœliac Gland.

Histological Changes.—The gland examination is in a condition of very advanced caseation and contains many small calcareous foci. Compared with the submaxillary gland, there is a more definite tendency to a demarcation of the caseous areas by fibrous tissue formation. Oxyphil leucocytes are plentiful; some of them are coarsely granular. There are several imperfectly formed giant cells. Specimens stain well by Pappenheim's method. Somewhat compressed and elongated cells which react well to this stain are not infrequently found, but there is no definite zone at the margin of the tubercular areas either of these cells or of the larger and

more spherical cells which react to Pappenheim's stain. Comparing the tubercular with the non-tubercular areas, it appears probable that all the cells present which take this stain are ordinary large lymphocytes, some of which have been modified by compression. The giant cell formations sometimes take up the pyronin stain slightly but never very intensely.

Distribution of Bacilli.—Tubercle bacilli are rather more plentiful than in the submaxillary gland, and sometimes occur in small groups.

SUMMARY.

The lungs contain many circumscribed, caseous tubercles. They are chronic in character and contain few bacilli.

VIRUS.—B I.
Animal Fed.—Pig 22.

Fed, when 10 weeks old, with 5,000,000 bacilli contained in the milk of Cow 64. Killed 111 days afterwards.

Lungs.

Histological Changes.—Large, caseous tubercles are present. They are not so numerous as in the lungs of Pig 20, but are similar in structure. The more advanced tubercles contain minute calcareous deposits.

Distribution of Bacilli.—Tubercle bacilli are rare.

SUMMARY.

Isolated, caseo-calcareous tubercles, containing few bacilli, are found in the lungs.

VIRUS.—B I.
Animal Fed.—Pig 24.

Fed, when 10 weeks old, with 5,000,000 bacilli contained in the milk of Cow 64. Killed 38 days afterwards.

Lungs.

Histological Changes.—Many small tubercles, containing dense masses of multinuclear leucocytes. The smallest tubercles are just commencing to caseate; in the larger the caseation is advanced.

Distribution of Bacilli.—Bacilli are present in the tubercles, but are very rare.

Mesenteric Glands.

Histological Changes.—In two small mesenteric glands examined there are no definitely tubercular lesions. The glands contain a moderately large number of multinuclear leucocytes.

Presence of Bacilli.—No tubercle bacilli have been found.

Ileo-Colic Gland.

The gland examined contains extensive, irregular tracts of caseation in which tubercle bacilli are moderately frequent. The tubercular areas are crowded with multinuclear leucocytes, and these cells are also present in large numbers in the rest of the tissue.

SUMMARY.

Several minute, probably retrogressive, tubercles are present in the lungs; they contain very few bacilli.

VIRUS.—B I.
Animal Fed.—Pig 26.

Fed, when 10 weeks old, with 10,000,000 bacilli contained in the milk of Cow 64. Killed 37 days afterwards.

Lungs.

Histological Changes.—Many small tubercles, similar in structure to those found in the lungs of Pig 24. In the non-tubercular areas patches of congestion are more frequent than in the lungs of Pig 24. Plasma cells are present but not numerous.

Distribution of Bacilli.—Tubercle bacilli are quite as rare as in the lungs of Pig 24.

Mesenteric Glands.

Histological Changes.—The two mesenteric glands examined show extensive and partially caseous tubercular areas. Both glands are crowded with multinuclear leucocytes of the finely granular type.

Distribution of Bacilli.—In the larger gland of the two, where the caseation is more advanced, a few bacilli have been found with difficulty. None have been found in the smaller.

Ileo-Cœcal Gland.

Histological Changes.—The gland examined contains large areas of partial caseation. Like the mesenteric glands, it is crowded with multinuclear leucocytes; many of these cells show a rather coarser granulation than in the mesenteric glands. Vascular channels are present in definitely affected areas.

Distribution of Bacilli.—Tubercle bacilli though not abundant and not occurring in groups are much more frequent than in the mesenteric glands.

SUMMARY.

Similar to Pig 24.

VIRUS.—B I.

Animal Fed.—Pig 30.

Fed, when 10 weeks old, with 1,000,000 bacilli contained in the milk of Cow 64. Killed 103 days afterwards.

Lungs.

Histological Changes.—In the specimen examined the capillaries are engorged with red corpuscles, and there are two small, early tubercles which are only slightly necrotic.

Distribution of Bacilli.—One or two bacilli have been found in the tubercles.

Cœliac Gland.

Histological Changes.—The gland is extensively affected, some areas being partially and others completely necrotic. The affected areas, where not necrotic, exhibit a well-marked development of cells of the endothelial type, with new capillary formation.

Distribution and Characters of Bacilli.—Bacilli, though not occurring in groups, are rather plentiful in most of the lesions. They measure from 2 to about $3 \cdot 5 \mu$. Both curved and beaded forms are common.

SUMMARY.

A few minute tubercles, containing very few bacilli, are present in the lungs.

VIRUS.—B I.

Animal Fed.—Pig 34.

Fed, when 7 or 8 months old, with 5,000,000 bacilli contained in the milk of Cow 64. Killed 46 days afterwards.

Mesenteric Gland.

Histological Changes.—In general character the condition of the gland resembles that of the coeliac gland of Pig 30, the only difference being that coarsely granular oxyphil leucocytes are present in rather large numbers.

Distribution of Bacilli.—Bacilli are scanty, much rarer than in Pig 30.

Ileo-Colic Gland.

Histological Changes.—The gland examined is much more extensively necrotic than the mesenteric gland.

Distribution of Bacilli.—In some of the caseous areas bacilli are very numerous.

SUMMARY.

There are plenty of bacilli in the ileo-colic glands, but, according to the post-mortem notes, there was little or no dissemination into distant organs.

VIRUS.—B I.

Animal Fed.—Pig 36.

Fed, when 7 or 8 months old, with 5,000,000 bacilli contained in the milk of Cow 64. Killed 115 days afterwards.

Lungs.

Histological Changes.—Small, circumscribed tubercles are present. They are bounded by a dense fibrous tissue zone and are centrally caseous.

Distribution of Bacilli.—No bacilli have been found.

Mesenteric Gland.

Histological Changes.—Compared with the mesenteric gland of Pig 34, the lesions are more advanced. In addition to large numbers of finely granular multinuclear leucocytes, coarsely granular eosinophil cells are present.

Distribution of Bacilli.—No bacilli have been found.

SUMMARY.

The lung contains isolated, retrogressive tubercles in which no bacilli have been found.

VIRUS.—B I.

Animal Fed.—Pig 38

Fed, when 7 or 8 months old, with 1,000,000 bacilli contained in the milk of Cow 64. Killed 109 days afterwards.

Lungs.

Histological Changes.—In the specimen examined there is one small tubercle. It is circumscribed by a dense fibrous zone. Its interior is filled with a mass of broken down multinuclear leucocytes.

Distribution of Bacilli.—No bacilli have been found.

Cœliac Gland.

Histological Changes.—Contains large caseous areas.

Distribution of Bacilli.—None found.

SUMMARY.

A minute, retrogressive lesion, containing no bacilli, is present in the specimen of lung.

VIRUS.—B I.

Animal Fed.—Pig 40.

Fed, when about 9 months old, with 10,000,000 bacilli contained in the milk of Cow 64. Killed 45 days afterwards.

Lungs.

In the specimen examined no tubercles or tubercle bacilli have been found. It is stated in the post-mortem notes that the lungs "showed one firm tubercle."

Ileo-Cæcal and Colic Glands.

An ileo-cæcal and a colic gland have been examined. Neither of them contain tubercles or tubercle bacilli.

SUMMARY.

No evidence of infection in the specimens examined.

VIRUS.—B I.

Animal Fed.—Pig 42.

Fed, when 9 months old, with 10,000,000 bacilli contained in the milk of Cow 64. Killed 117 days afterwards.

Lungs.

The specimen of lung selected for examination proved to be normal. At the post-mortem examination the lungs were found to contain " about 10 small tubercles."

Mesenteric Glands.

The gland examined shows advanced caseation. with some calcareous points, and contains a few bacilli.

SUMMARY.

The specimen of the lungs is normal. The mesenteric gland shows advanced caseation and contains a few bacilli.

VIRUSES.—B I. and B II.

Animal Fed.—Pig 2.

Fed for 21 days with tuberculous milk from Cow 500 (B II.), Cow 4 (B II.), and Cow 18 (B I.). Age at commencement of experiment, six weeks. Killed 160 days afterwards.

Lungs.

Histological Changes.—The specimens examined contain numerous tubercles. The largest are about 2 mm. in diameter and the smallest about a quarter of the area of the microscopic field (low power). All except the smallest are in a condition of advanced caseation, and some of them contain minute calcareous points. Some of the smallest tubercles are only slightly necrotic, occur in groups, and are partially confluent; but with these exceptions, the tubercles occur as isolated nodules, clearly marked off from the surrounding tissue, and bounded by a broad fibrous zone; except where completely necrotic, the tubercles are well supplied with blood capillaries.

Large masses of multinuclear leucocytes are found in the interior of all the tubercles, and these cells are also present as an infiltration in the peripheral zone. The framework of the outer areas of the tubercles consists of epithelioid cells and fibroblasts. It appears probable, particularly in the smallest and least advanced tubercles, that these cells are, to a very large extent, of endothelial origin. Even in the largest tubercles the number of cells which react to van Gieson's stain for fibrous tissue is small. There is a deposit of fibrin in some of the blood-vessels, but none in the tubercles.

Presence of Bacilli.—Bacilli have been found, but are extremely rare.

SUMMARY.

The lung contains numerous tubercles which are of a chronic type and not obviously progressive. Bacilli are very rare.

VIRUS.—B IV.

Animal Fed.—Pig 58.

Fed when 6 or 7 months old, with 10,000 bacilli contained in the milk of Cow 164. Killed 43 days afterwards.

Mesenteric Gland.

Histological Changes.—The gland examined exhibits extensive and irregular areas of caseation with many giant cells. Oxyphil leucocytes are present in enormous numbers. They are all of the finely granular variety.

Distribution and Characters of Bacilli.—Bacilli are numerous in all the lesions and are often collected in groups. They measure from 2 to 3μ in length, are generally straight, and sometimes beaded.

SUMMARY.

The dose, though small, has succeeded in multiplying in the mesenteric glands.

VIRUS.—B IV.

Animal Fed.—Pig 80.

Killed 131 days after feeding with emulsion of tissues of G.P. inoculated from Cow 164. Dose 10,000,000 bacilli. Age, at commencement of experiment, 7 or 8 weeks.

Lungs.

Histological Changes.—The greater portion of the tissues in the sections examined consists of large irregular areas of complete consolidation, in which the alveoli are entirely obliterated. Bordering upon these areas, the alveoli are almost completely filled with desquamated epithelium. Interspersed between the affected regions, small tracts of normal alveoli are found. The consolidated areas are extensively necrotic.

The nuclear remains of multinuclear leucocytes are present in the necrotic areas. In the outer portion of the consolidated areas there are some small groups of multinuclear leucocytes which retain an oxyphil stain. Small lymphocytes are numerous, and in some places there is a definite infiltration of these cells. Plasma cells are present and stain well. They form a well-defined, but not dense ring round some of the bronchioles; here they exhibit typical varieties of form (excentric nucleus; protoplasm sometimes circular in outline, but more frequently oval, elongated, or rectangular). Groups of plasma cells exhibiting the same features are also found sometimes at the periphery of consolidated areas. In other situations, amongst the consolidated tissue, small groups of the circular variety of cell, indistinguishable from ordinary large lymphocytes, are present. Individual cells of this last type are also found not infrequently in the walls of alveoli to which the process of consolidation has not extended. Cell fusions or imperfectly formed giant cells are rather frequent. In the consolidated areas there has been extensive destruction of elastic tissue. No fibrin is present.

Distribution of Bacilli.—Bacilli are scanty and do not occur in groups.

Liver.

Histological Changes.—A piece was selected for examination which contained minute grey tubercles, just visible to the naked eye. These tubercles, though involving the liver parenchyma, always border upon

the margin of a lobule. Several of the lesions are continuous with, and apparently arise from, the tissues within Glisson's capsules.

The frame-work of the tubercles consists of an irregular network of cells, apparently of endothelial origin, and, amongst these, the remains of liver cells. Multinuclear leucocytes and small lymphocytes are present within these structures in about equal numbers. Amongst all the tubercles several typical plasma cells are to be found. These cells also occur, in small numbers, in the interlobular tissue which shows no evidence of tubercular change.

Distribution of Bacilli.—Bacilli are very rare, but one or two have been found within tubercles.

SUMMARY.

Disseminated, progressive tuberculosis, with particularly severe incidence on the lungs. Bacilli scanty.

VIRUS.—B IV.
Animal Fed.—Pig 92.

Fed, when 8 weeks old, with 10,000,000 bacilli contained in milk of Cow 172. Killed, when moribund, 137 days afterwards.

Lungs.

Histological Changes.—The tissue examined is in a condition of nearly complete consolidation. In the more advanced areas the central parts are necrotic and the alveoli are completely obliterated. Where the process is rather less advanced, remains of alveoli, filled with cellular material can be seen. Irregularly distributed amongst the consolidated tissue are small groups of alveoli which are less extensively affected. Many of these exhibit an acute catarrhal process; the alveolar walls are thickened and the alveolar lumen is filled with coagulated material. Very few alveoli are found which show no pathological change. The pleural surface is thickened and infiltrated.

Typical coarsely granular eosinophil cells are numerous and are often massed together in groups. These groups occur at the margin of consolidated areas, but not in situations where there is advanced necrotic change. The majority of these cells are nearly twice the size of the ordinary finely granular multinuclear leucocyte, but some of them are no larger than the latter type of cell. The nuclei of the coarsely granular cells are either circular in outline, or oval, or slightly indented; some of them take a deep stain with methylene blue, like the nuclei of the finely granular type, but many of them only take a pale blue stain; the pale nuclei are generally larger than the deeply stained nuclei. The affinity of these cells for eosin is much stronger than that of the finely granular leucocytes. The latter cells are also numerous, particularly in situations where necrosis is commencing or advanced, but only take a faint pink, almost homogeneous, protoplasmic stain, which is in marked contrast to the deep, brilliant stain of the coarsely granular cells. Plasma cells, or large lymphocytes are numerous; they are irregularly distributed, and occur in situations where necrotic change has not commenced. There is marked destruction of elastic tissue. There are some small deposits of early fibrin, generally within or near situations where there is a catarrhal exudate into the alveoli.

Distribution and Characters of Bacilli.—As seen under a low power, the section is irregularly dotted with red points, consisting of groups of bacilli. These groups very frequently occur amongst detached cellular material lying free within alveoli. Under a higher magnification bacilli are found distributed in large numbers throughout all parts of the tissue. The coarsely granular eosinophil cells do not exhibit any particular relationship to the bacilli. In sections stained by carbol-fuchsin followed by strong methylene blue, without any intermediate washing in acid or other liquid, both the coarsely granular oxyphil cells and the bacilli are demonstrated. Groups of oxyphil cells do not occur in the same situations as groups of bacilli, nor is there any appearance which suggests that the oxyphil cells are attacking the bacilli. The bacilli are from 2 to 5 or 6 in length; about half of them are curved, and nearly as many as one-third are regularly beaded.

Liver.

Histological Changes.—The liver is crowded with minute miliary tubercles, several being present within each lobule. These tubercles frequently border upon or partially involve the interlobular tissue, but many of them are entirely intralobular.

There is an infiltration of coarsely granular eosinophil cells, of the same type as those found in the lung, throughout the interlobular tissues. These cells are infrequent in the interior of the lobules. They bear a definite association to the tissue within Glisson's capsules and to the interlobular spaces rather than to the tubercles. Ordinary multinuclear leucocytes are present within all the tubercles; the majority of them are partially disintegrated and retain little or no protoplasmic stain. Small lymphocytes are abundant, both in the interstitial tissue and in the tubercles. Plasma cells are present but rare; most of them are found in the interstitial tissue. The framework of the tubercles consists of cells, probably of endothelial origin, which have increased irregularly, sometimes fused together into imperfectly formed giant cells, and to a greater or less extent obliterated the parenchymatous cells. There is a delicate network of fibrin within many of the tubercles and within some of the blood vessels.

Distribution of Bacilli.—Bacilli are present in most of the tubercles. The number visible in each tubercle varies from about two or three to about a dozen.

Tracheal Lymphatic Gland.

Histological Changes.—This gland is in a condition of very advanced necrosis. Amongst the portions of surviving glandular tissue are large numbers of coarsely granular eosinophil cells.

Distribution of Bacilli.—Bacilli are rather plentiful, but never occur in groups.

SUMMARY.

Acute, disseminated infection, with particularly severe incidence on the lungs. Bacilli very abundant in the lungs and fairly numerous in the liver.

VIRUS.—B IV.
Animal Inoculated.—Pig 94.

Killed 61 days after subcutaneous inoculation with 1 mg. of culture of B IV. (direct). Age at commencement of experiment, ten weeks.

Lungs.

Histological Changes.—The lung is in a transitional stage between miliary tuberculosis and commencing generalised consolidation. Continuous with small miliary tubercles are alveoli which have thickened, infiltrated walls and a lumen occupied by desquamated epithelium and leucocytes; and here and there alveoli have become completely obliterated. In this way irregular tracts of partial consolidation are produced. The intervening tissue is slightly emphysematous.

Multinuclear leucocytes, all of the finely granular type, are numerous and generally distributed. Small lymphocytes are rather less numerous. Both types of cells are more definitely collected in areas of consolidation. Plasma cells are present but rare, and nowhere form large groups. In all the infiltrated and consolidated areas endothelial cells play a prominent part in the new tissue formation. No fibrin is demonstrable. There is some destruction of elastic tissue, but only slight.

Distribution and Characters of Bacilli.—Bacilli are very numerous throughout the tissue. They are particularly conspicuous in alveoli where the epithelium has desquamated. In these situations they appear to have multiplied within epithelial cells. The bacilli are from 1·5 to 4 μ in length, sometimes, but not very frequently, curved and occasionally beaded.

Liver.

Histological Changes.—The liver is in a condition of disseminated tuberculosis. Most of the lesions are small, in an early stage, and not caseous. They frequently contain giant cells. The lesions are so numerous throughout the tissue that the general appearance is that of a diffuse tubercular infiltration, which is not separable into discrete tubercles. The intralobular tissue, as well as the interstitial tissue, is permeated by this infiltration.

The following are the main details of histological interest —(1.) *Eosinophil Leucocytes.* These are numerous in all the lesions ; they are all of the finely granular type ; very few of them are disintegrated or have lost their protoplasmic stain. (2.) *Small Lymphocytes.* These cells are about as numerous as the leucocytes, and occur in association with the latter. (3.) *Large Lymphocytes.* Only small numbers of these cells are present. (4.) *Plasma Cells.* Cells which react to Pappenheim's stain, but are morphologically different from ordinary large lymphocytes occur, but with rarity ; the possibility must be held in view that those which are present are merely lymphocyes modified by compression. (5.) *Liver Cells.* At the margin of tubercular areas a few mitotic figures have been found within liver cells. Fragmentation of the nuclei of these cells is also occasionally seen. Within the tubercles multiplication of the nuclei of these cells without division of their protoplasm is common. The greater number of the giant cells, both from the character of their nuclei and the staining reaction of their protoplasm, appear to have originated from liver cells. (6.) *Endothelial Cells.* In some of the tubercles, particularly those where there are definite signs of an increase of liver cells, the endothelial cells show no definite increase ; they are irregular in outline, somewhat swollen, and their nuclei stain faintly. In other situations, however, there is clear evidence that the endothelial cells are taking an active part in the tubercular process. In these latter situations the endothelial cells are swollen, take a deep nuclear stain, are definitely increased in numbers, and have often fused together into more or less typical giant cells. Situations characterised by this activity of endothelial cells are not confined to the interlobular tissue, but also occur in tubercles situated in the interior of lobules. In the intralobular tubercles where the increase of endothelial cells is prominent, the increase of parenchymatous cells is usually less prominent. It appears probable, with regard to the intralobular tubercles, that the activity and nuclear division of the liver cells marks an earlier stage, which does not persist for long, and is followed by a gradual disappearance of the liver cells, accompanied by active increase on the part of the endothelial cells. (7.) *Fibrin.* No fibrin is present.

Distribution and Characters of Bacilli.—Most of the tubercular areas, both within the lobules and in the interstitial tissue, contain several bacilli. The bacilli generally occur singly, and are frequently within giant cells. They are from 2 to 3μ in length, generally straight, stain somewhat irregularly, but are rarely definitely beaded.

Mesenteric Gland.

Histological Changes.—Extensive necrotic areas. Many multinuclear leucocytes, but most of them disintegrated.

Distribution and Characters of Bacilli.—Bacilli are extremely numerous. In some situations, at the margin of necrotic areas, they are packed together in masses of small colonies. They vary in length from 2·5 to 4μ. The majority are straight, but the longest forms are generally curved. A small proportion are regularly beaded.

Summary.

The lung is in a transitional stage of miliary tuberculosis advancing to generalised consolidation. It contains very large numbers of tubercle bacilli. The liver is in a condition of diffuse tubercular infiltration.

VIRUS.—B IX.
Animal Fed.—Pig 108.

Killed 113 days after being fed with ·1 mg. of culture of B IX. Age, at commencement, 15 weeks.

Lungs.

The specimen contains a group of tubercles which occupy the greater portion of the tissue. The majority of the individual tubercles are bounded by a broad fibrous zone. In their interior they contain dense masses of multinuclear leucocytes, and often they have commenced to caseate. Bacilli are very rare.

Liver.

The specimen contains two tubercles which have a broad fibrous periphery and internal to this a zone of epithelioid cells. The tubercles from their periphery to their centre are densely infiltrated with lymphocytes and leucocytes. No bacilli have been found in them.

Kidneys.

The specimen examined is normal.

Summary.

The lungs and liver contain circumscribed tubercles of a retrogressive type. Bacilli are very rare.

VIRUS.—B IX.
Animal Fed.—Pig 114.

Killed 48 days after being fed with 1 mg. of culture of B IX. Age, at commencement, 14 weeks.

Lungs.

The lesions are similar to those found in the lungs of Pig 108. Tubercle bacilli are present, but very rare.

Liver.

Three small tubercles are present in the specimen. They are identical in histological characters with those found in the liver of Pig 108. In one of them a tubercle bacillus has been found.

Kidneys.

The specimen examined contains a small, suspicious focus of infiltration, in which no bacilli are present, but is otherwise normal.

Portal Gland.

The gland contains many tubercular foci which are slightly caseous. No bacilli have been found.

Summary.

Disseminated tubercles of a retrogressive type, and containing very few bacilli, are present in the lungs and liver. The lesions are similar to those found in Pig 108.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H 56. F.T.

Animal Inoculated.—Pig 21.

Pig 21 was inoculated subcutaneously with 1 mg. of culture of H 56. F.T., and was killed 98 days afterwards.

Lungs.

Specimens from two parts of the lungs show groups of circumscribed, caseous, and obviously retrogressive tubercles. They contain in or near their centres large numbers of finely granular multinuclear leucocytes, and at their periphery, which is densely fibrous, coarsely granular leucocytes are found. No tubercle bacilli have been found.

Liver and Kidneys.

No lesions or bacilli have been found in the specimens of these organs.

SUMMARY.

Retrogressive tubercles are present in the lungs.

VIRUS.—H 56. F.T

Animal Inoculated.—Pig 23.

Pig 23 was inoculated subcutaneously with 50 mg. of culture of H 56 F.T., and was killed 65 days afterwards, when apparently well.

Lungs.

The tissue shows marked thickening of the alveolar walls and contains numerous tubercles. These tubercles are slightly caseous in the centre and have a broad cellular zone which consists of a reticulum of fibroblasts infiltrated by numerous leucocytes and lymphocytes and a sprinkling of plasma cells. Many of the leucocytes are coarsely granular. Tubercle bacilli are present in some of the tubercles, but are very rare.

Liver.

Many discrete tubercles are present. In these the liver cells are more or less completely obliterated and the lesions are composed of a reticulum of fibroblasts and endothelial cells, amongst which there is an infiltration of lymphocytes, leucocytes, and, in scanty numbers, plasma cells. Many of the leucocytes are coarsely granular. There are several giant cells. Tubercle bacilli are present, but are extremely rare.

Kidneys.

No lesions or tubercle bacilli have been found.

SUMMARY.

Numerous tubercles are present in the lungs and liver. The tubercles contain very few bacilli and exhibit marked evidence of conservative changes.

C. THE PROCESS OF INFECTION.

Dr. Griffith has inoculated a series of pigs with cultures of B IV., and killed them at intervals of 1, 3, 5, 8, and 14 days after inoculation. Each animal was inoculated subcutaneously in the ear, and received a dose of 20 mg. The following are the results of my microscopic examinations of the tissues of these animals. The statement that bacilli are absent in any particular organ means that none have been found in three sections which have been completely searched with the aid of the mechanical stage.* The cervical glands referred to are the glands nearest to the sites of inoculation.

Pig 144 (1 day).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Lungs - - - - -	Absent
Liver - - - - - -	Absent
Kidneys - - - - -	Absent
Spleen - - - - -	Absent
Bronchial Gland - - -	Absent
Cervical Gland - - - -	Present

Pig 146 (3 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Lungs - - - - -	Absent
Liver - - - - - -	Absent
Kidneys - - - - -	Absent
Spleen - - - - -	Absent
Bronchial Gland - - -	Absent
Cervical Gland - - - -	Present

Pig 148 (5 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Lungs - - - - -	Present
Liver - - - - - -	Absent
Kidneys - - - - -	Absent
Spleen - - - - -	Absent
Bronchial Gland - - -	Absent
Cervical Gland - - - -	Present

* My laboratory attendant, Mr. Nicholls, has searched these specimens for me.

Pig 142 (8 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Lungs - - - - - -	Present
Liver - - - - - -	Present
Kidneys - - - - -	Absent
Spleen - - - - - -	Present
Bronchial Gland - - - -	Present
Cervical Gland - - - -	Present

Pig 150 (14 days).

Dissemination of Bacilli.

Tissue.	Presence or Absence of Bacilli in the Sections examined.
Lungs - - - - - -	Present
Liver - - - - - -	Present
Kidneys - - - - -	Absent
Spleen - - - - - -	Present
Bronchial Gland - - - -	Present
Cervical Gland - - - -	Present
Mesenteric Gland - - -	Present
Portal Gland - - - -	Present

Microscopic Details.

Lungs.

The 5 days' specimen is the earliest in which bacilli have been found. In this specimen a group of four or five bacilli has been found with difficulty. They are not associated with any definite tubercle formation. In the 8 days' specimen bacilli are fairly numerous and are irregularly distributed in all parts of the tissue. Many of the situations in which they occur are expanding into definite tubercles. In one of the largest of these foci about sixty bacilli are present, but usually the number is less, varying from about twenty to three or four. In the 14 days' specimen bacilli are present in fairly large numbers but are not more numerous than in the 8 days' specimen. They generally occur within definite tubercles. Comparing these tubercles with earlier tubercles, of about the same size, in the 8 days' specimen, it is frequently found that bacilli are scantier in the older tubercles. There is evidence, therefore, that between the 8th and the 14th day the bacilli have not been able to multiply freely, and that some of them have been destroyed.

In the 1 and 3 days' specimens finely granular oxyphil leucocytes occur in fairly large numbers in all parts of the tissue, but are nowhere aggregated into definite groups; no coarsely granular forms have been noticed. Perhaps the leucocytes are rather more numerous in the 3 than in the 1 days' specimen. In the 5 days' specimen there is again some general increase in the number of leucocytes, but no groups have been noticed; an occasional coarsely granular cell is present. In the 8 days' specimen the leucocytes are not only generally distributed in moderately large numbers, but are also aggregated into groups; these groups occur where tubercle bacilli are present; all the leucocytes are of the finely granular type. In the 14 days' specimen there is again a decided increase in the leucocytes; they form much larger groups and many of the cells are partially disintegrated; these groups of leucocytes are particularly noticeable within the tubercles; they are all of the finely granular type.

In the 1 and 3 days' specimens plasma cells are found in all parts of the sections; they generally occur singly and do not form groups. In the 5 days' specimen these cells are more numerous and sometimes occur in small groups. In the 8 days' specimen there is again a definite increase in the number of plasma cells and the groups which they form are larger; these cells are particularly noticeable in the early tubercles. In the 14 days' specimen the plasma cells exhibit more variety of shape than in the 8 days'; compressed and elongated forms are more numerous; the total number of these cells does not appear to be greater than in the 8 days' specimens.

In the early tubercles present in the 8 days' specimen, desquamated epithelial cells are still recognisable; the endothelial cells are conspicuous and swollen, and there are many small lymphocytes; there are some cell fusions but no definite giant cells. In the 14 days' tubercles very few epithelial cells are recognisable, but cells resembling endothelial cells, or young fibroblasts, are more numerous and there are a few giant cells; no fibrin is present.

Livers.

In the 8 and 14 days' specimens bacilli are rare; a few only have been found in each case. They occur within small tubercles, which are present in both specimens, but are not numerous. In the 8 days' specimen there are four tubercles, two within lobules and two at the periphery of lobules. These tubercles show partial obliteration of the parenchymatous cells and are infiltrated with leucocytes, lymphocytes, and plasma cells. In the small tubercles found in the 14 days' specimen the parenchymatous cells are completely obliterated, and cells resembling endothelial cells or young fibroblasts are much more numerous; a few giant cells occur. This specimen also contains a much larger lesion, which is completely necrotic in the centre and is surrounded by a well-marked fibrous zone; external to this lesion there are some small foci which are filled with lymphoid cells. No tubercle bacilli have been found either in the necrotic lesion or in the surrounding foci.

Kidneys.

No tubercles or tubercle bacilli have been found.

Spleens.

No definite lesions have been found, but in the 8 and 14 days' specimens a few bacilli have been seen (3 or 4 in each).

Bronchial Glands.

In the 8 days' specimen there are no definite lesions, but a few bacilli have been found. In the 14 days' specimen there are several early lesions in each of which a few bacilli are present.

Cervical Glands.

In the 1 day specimen bacilli are scattered in small groups throughout all parts of the gland. The bacilli are identical in every respect with serum grown bacilli. The bacilli are not associated with any definite lesions. In the 3 days' specimen the bacilli are much more numerous, many of them are distinctly longer than serum grown bacilli; in many places these are large collections of multinuclear leucocytes in association with the bacilli. In the 5 days' specimen they are still more numerous, the longer forms and the forms identical with serum grown bacilli being about equal in number. There are large collections of multinuclear leucocytes, and the fragments of these cells, in association with the bacilli; and in many places where the bacilli are numerous the lymph cells have disappeared. In the 8 days' specimen the bacilli have again increased greatly in numbers. Both the serum grown forms and the longer bacilli are about equally numerous. It is evident that absorption of the inoculated material is still rapidly going on. Multinuclear leucocytes, many of them broken up, are collected in very large numbers in the situations where the bacilli are most numerous, and large tracts of commencing caseation are appearing. In the 14 days' specimen the gland is very extensively caseous, and is swarming with bacilli which have often aggregated into small groups, visible everywhere with a low power. The bacilli are evidently multiplying.

In the 1 day specimen coarsely granular leucocytes are scattered in large numbers throughout the section; finely granular leucocytes are also fairly numerous in many situations, but are not closely aggregated together.

In the 3 days' specimen coarsely granular oxyphils are still fairly numerous, but not so abundant as in the 1 day specimen; on the other hand the finely granular oxyphils are distinctly more numerous. In the 5 days' specimen there is found a progressive change in the same direction, the coarsely granular cells again showing a diminution, and the finely granulars some increase in numbers. In the 8 days' specimen the same progressive change is noticeable; many of the finely granular cells have now become disintegrated. In the 14 days' specimen some coarsely granular cells are still present, but in many cases cells probably belonging to this type give a brilliant oxyphil stain, but have lost their sharp granulation. Finely granular leucocytes, a large proportion of them disintegrated, are extremely numerous.

Mesenteric Gland.

In the mesenteric gland, belonging to the 14 days animal, no lesions are present. One bacillus has been found, after searching three specimens.

Portal Gland.

In the portal gland belonging to the 14 days' animal there are several early lesions, in most of which one or two bacilli are present.

D. SUMMARY.

Disseminated tuberculosis has been readily produced by feeding, and it is noteworthy that the lungs are generally affected. The lesions produced in these feeding experiments are in the majority of cases discrete and of a chronic type, being sometimes apparently retrogressive and sometimes slowly progressive. Less frequently, a much more extensive disease has been produced, and the lesions are characteristic of a severe infection. In all the feeding experiments on which I have reported, bacilli of bovine origin have been administered

Attention may be called to the characters of the lesions found in two animals which were inoculated subcutaneously. Pig 94 received 1 mg. of a virus (B IV.) known to be highly virulent to bovines and rabbits, and Pig 23 received 50 mg. of a virus (H. 56. F.T.) known to be of much lower virulence for these two classes of animals. In both pigs disseminated tuberculosis was produced, but the small dose of B IV. produced a distinctly severer type of disease than the large dose of H. 56. F.T. But in Pig 23 the tubercles were more numerous than is usually the case in calves which have been inoculated with the same dose of a virus possessing a relatively low degree of virulence for the bovine. On the provisional assumption that the results of these two experiments may be regarded as typical, the pig appears, like bovines, monkeys, and goats, to be highly susceptible to the more virulent bacilli, but to exhibit against bacilli of lower virulence a power of resistance which is rather less than that possessed by the calf, but very much higher than the resistance of anthropoid apes and monkeys.

My histological study of "the process of infection" in pigs shows how rapidly the dissemination of bacilli takes place after subcutaneous inoculation, and may be compared with the investigation which I have reported above on the early dissemination of bacilli in the bovine. These results show that the old theories concerning the resistance offered by the lymphatic glands to the spread of tuberculosis need revision.

EXPERIMENTS ON DOGS.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B IV.
Animal Inoculated.—Dog 26.

Dog 26 received an intraperitoneal inoculation with an emulsion of organs of guinea-pigs which was estimated to contain about ten million bacilli. The animal was killed after 71 days.

Lungs.

The tissue is crowded with miliary tubercles. These are partially caseous, irregular in outline, and show no fibrous demarcation. Their periphery is densely infiltrated with leucocytes and lymphocytes, and contains a few plasma cells. The plasma cells occur singly, here and there; they nowhere form a definite peripheral zone. There are no giant cells. No fibrin is demonstrable. Tubercle bacilli are abundant in all the lesions. The great majority of them are short and straight.

Liver.

The liver is permeated with irregular areas of partial necrosis. These have not the structure of organised tubercles, but consist merely of a central area containing partially necrotic liver parenchyma and an irregular peripheral zone of leucocytes and lymphocytes. No fibrin is demonstrable. Bacilli are present in all the lesions, but are less numerous than in the lungs, and longer forms are more frequent.

Kidneys.

The kidneys contain small tubercles in which the renal cells are only partially obliterated. These foci are infiltrated with leucocytes and lymphocytes, and are partially surrounded by plasma cells. Bacilli are present in the lesions, but are scanty.

Mediastinal Gland.

The gland is permeated with pale, irregular areas, in which the lymph cells stain faintly, and are infiltrated with multinuclear leucocytes. In most of these areas the outline of the cells is still definitely recognisable, but here and there the cells have completely broken down and formed a caseous focus. Between these pale areas there are dense groups of cells which have the variety of shape and the staining reaction of typical plasma cells. No fibrin is present. Bacilli are present in all parts of the gland, and are very abundant in the situations which stain faintly. The great majority of the bacilli are intracellular. Within the large mononuclear cells (lymphocytes and endothelial cells), they have grown into clusters, and sometimes have replaced the cell by a solid mass of bacilli. A bacillus is often seen attached to or within a multinuclear leucocyte, but the bacilli associated with these cells rarely form groups, and it is evident that close proximity to multinuclear leucocytes is unfavourable to their growth. The majority of bacilli are notably longer than in the lung. The main features of this specimen, therefore, are (1) the small amount of tissue destruction in proportion to the enormous number of bacilli present, and (2) the marked tendency of the bacilli to reside and multiply within cells.

Summary.

Progressive, disseminated tuberculosis of a severe type. The disease appears to be most acute in the lungs and liver. The bacilli have been able to multiply actively within the cells of the lymph gland examined, but have not produced much tissue destruction.

VIRUS.—B IV.
Animal Inoculated.—Dog 74.

Dog 74 was inoculated subcutaneously with 10 mg. of culture isolated from Dog 56 and was killed 63 days afterwards.

Lungs.

The specimen contains several small tubercles which are not caseous, but are densely infiltrated with lymphocytes and contain a relatively small number of leucocytes. Many of the cells within the tubercles react well to Pappenheim's stain. The lesions are obviously retrogressive. Tubercle bacilli are present in the lesions but are extremely scanty.

Liver and Kidneys.

No lesions or tubercle bacilli have been found.

Submaxillary Lymphatic Gland.

A few small slightly caseous foci are present. In these a few tubercle bacilli have been found.

Summary.

The lungs contain small, obviously retrogressive lesions, in which very few bacilli can be found. The other organs examined show little or no evidence of tuberculosis.

VIRUS.—B IV.
Animal Fed.—Dog 92.

Dog 92 was fed with a dose of 25 mg. of culture isolated from Dog 54, and was killed 64 days afterwards.

Lungs.

The specimen contains a small tubercle which is centrally caseous and possesses a broad cellular periphery composed chiefly of lymphocytes and plasma cells. In the caseous material within the tubercle, bacilli are fairly numerous; none have been found elsewhere.

Liver, Kidneys, and Mesenteric Gland.

The specimens examined of these three organs show neither tubercles nor tubercle bacilli.

Summary.

Tubercles of the chronic type are present in the lungs, but none have been found in the other organs examined.

VIRUS.—B IX.
Animal Fed.—Dog 58.

Dog 58 (pup) was fed with a dose of 1 mg. of culture of B IX. and was killed 49 days afterwards.

Lungs.

The specimen contains a caseous tubercle which has a broad periphery in which fibroblasts, lymphocytes, and plasma cells are abundant. In this tubercle a few bacilli are present, but none have been found elsewhere.

Liver and Kidneys.

No lesions or tubercle bacilli have been found in the specimens examined.

Mesenteric Gland.

Several caseous foci are present. In some of these tubercle bacilli are moderately numerous.

SUMMARY.

The mesenteric glands and the lungs contain tubercles of the chronic type.

VIRUS.—B IX.
Animal Inoculated.—Dog 114.

Dog 114 (pup) received an intraperitoneal inoculation with 1 mg. of culture of B. IX. and died 32 days afterwards.

Lungs.

The specimen of lung is highly vascular and almost completely solidified, owing to the presence of enormous numbers of multinuclear leucocytes. There are no lesions suggestive of tuberculosis. No tubercle bacilli have been found.

Liver.

Miliary tubercles are very numerous. They are invaded with multinuclear leucocytes and contain many tubercle bacilli.

Kidneys.

Beneath the capsule and passing inwards for a short distance is a narrow area which is densely infiltrated with leucocytes, lymphocytes, and plasma cells. It contains numerous bacilli. The bacilli are identical in appearance with bacilli grown on serum.

Cœliac Gland.

There is a large necrotic patch at the margin and numerous foci of early caseation are present in the interior. Bacilli are very abundant. This gland has evidently absorbed a considerable portion of the inoculated material.

SUMMARY.

Miliary tuberculosis of the liver.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS—H 8. S.C.
Animal Inoculated.—Dog 11.

Dog 11 was inoculated subcutaneously with 1 mg. of culture of H8. S.C., and was killed 92 days afterwards.

Lungs.

In one specimen a small tubercle has been found. It is slightly caseous in the centre and is bounded by a very dense zone of cells consisting mainly of fibroblasts and lymphocytes. No tubercle bacilli have been found.

SUMMARY.

The lungs contain definite, but obviously retrogressive tubercles.

VIRUS.—H 25. A.T.
Animal Fed.—Dog 1.

Dog 1 was fed with 10 mg. of culture of H 25. A.T., and died 94 days afterwards.

Lungs.

The tissue shows a good deal of catarrhal exudate, and contains a patch of complete consolidation. This patch is not necrotic but is infiltrated with leucocytes, lymphocytes and plasma cells. No fibrin is present. Tubercle bacilli are moderately numerous in the consolidated area.

Liver, Kidneys, and Mesenteric Glands.

The specimens of these organs which have been microscoped show neither tubercles nor tubercle bacilli.

SUMMARY.

The lungs show patches of consolidation due to the presence of tubercle bacilli.

VIRUS.—H 25. A.T.
Animal Fed.—Dog 3.

Dog 3 was fed with 1 mg. of culture of H 25. A.T., and was killed 191 days afterwards.

Suspicious foci from the lungs, liver, and kidneys have been microscoped. They have not the appearance of genuine tubercles; no tubercle bacilli have been found in them. A mesenteric gland has also been examined and has been found to be normal.

SUMMARY.

No microscopic evidence of tuberculosis.

VIRUS.—H 63. G.R.

Animal Inoculated.—Dog 15.

Dog 15 was inoculated intraperitoneally with 10 mg. of culture of H 63. G.R. and died 48 days afterwards.

Lungs.

The lungs are highly congested and contain many small foci which are irregular in outline, not circumscribed, not caseous, and not typical of tuberculosis. These foci are filled with leucocytes and lymphocytes. No fibrin is present. The foci referred to are swarming with tubercle bacilli. Bacilli are also present in other situations which show no definite histological lesions. The bacilli vary in length from $1 \cdot 5$ to $4 \cdot 5\mu$; they are often curved and often stained irregularly.

Liver.

The sections show a network of lightly staining ramifying tracts, between which islands of unaltered liver tissue have survived. In these tracts, which are not caseous, the parenchymatous cells have disappeared, or at least have lost their characteristic appearance ; cells with pale, elongated, irregularly outlined nuclei are very numerous ; and both small lymphocytes and multinuclear leucocytes are present, the former being more numerous than the latter. No fibrin is present. The areas referred to are swarming with clusters of tubercle bacilli. Bacilli are also present, though rarely, in the blood-vessels.

Kidneys.

No tubercles have been found, but a few small groups of tubercle bacilli are present in the interstitial tissue.

Bronchial Gland.

Extensively necrotic and swarming with colonies of tubercle bacilli. No fibrin.

SUMMARY.

The tissues are swarming with tubercle bacilli,which have produced a relatively small amount of tissue destinction. No fibrin.

C. SUMMARY.

When the bacilli are administered by feeding they readily gain access to the lungs, and there set up lesions which are usually not of a very severe type. A much more acute type of disease has been produced by intraperitoneal inoculation both in Dog 26, which was inoculated with a typical bovine virus, and in Dog 15, which was inoculated with a human virus of low virulence for the bovine.

Most of the feeding experiments have been performed with bovine bacilli of high virulence, but it is noteworthy that in one case feeding with human bacilli (H 25. A.T.) of relatively low virulence for the bovine produced disseminated disease of moderate severity. The dog, therefore, affords another illustration of the identity in kind of the various bovine and human viruses which have been investigated.

In some specimens the bacilli have multiplied very abundantly, but with the production of a relatively small amount of tissue destruction.

EXPERIMENTS ON CATS.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Cat 26.

Cat 26 was inoculated subcutaneously with 1 mg. of culture of B I., and was killed 35 days afterwards.

Lungs.

The tissue is in a condition of partial consolidation. The consolidated foci have an irregular, patchy distribution throughout the tissue. They are slightly necrotic, and contain masses of multinuclear leucocytes. Tubercle bacilli are distributed throughout the tissue and are plentiful in the consolidated foci; they are frequently present within desquamated epithelial cells.

Liver.

The liver is crowded with minute, miliary tubercles. The lesions are centrally caseous and have a peripheral zone of lymphocytes and leucocytes, but show no evidence of fibrous demarcation. In nearly all of the lesions a few tubercle bacilli can be seen.

Bronchial Gland.

The gland is permeated with numerous caseous tracts Bacilli are scattered about these areas in small numbers but show no evidence of active multiplication.

SUMMARY.

Acute, disseminated tuberculosis.

VIRUS.—B III.

Animal Inoculated.—Cat 32.

Cat 32 (kitten) was inoculated subcutaneously with 1 mg. of culture of B III., and died 17 days afterwards.

Lungs.

No tubercles are present, and, with the exception of a little congestion and thickening of the alveolar walls, the general appearance of the tissue is normal. No fibrin is present. Throughout the tissue the alveolar walls are swarming with tubercle bacilli. The bacilli are evidently multiplying freely, as they nearly always occur in small groups or colonies.

Liver.

No histological change is noticeable. Bacilli are present in all parts of the tissue, but are much less numerous than in the lungs. Where they occur they generally have grown into a small cluster.

Kidneys.

There are no histological lesions, but bacilli are present throughout the tissue. Where they occur, they generally form small groups. These groups occur both in the glomeruli and in the interstitial tissue.

Inguinal Gland.

The gland shows extensive caseating tracts which contain numerous multinuclear leucocytes and the nuclear fragments of these cells. Bacilli are present in enormous numbers, and in many parts of the section form large, continuous masses which have almost obliterated the tissue elements.

SUMMARY.

All the organs are overrun with tubercle bacilli, but, except in the inguinal gland, there is little or no tissue change.

VIRUS.—B III.

Animal Inoculated.—Cat 34.

Cat 34 received an intraperitoneal inoculation with 1 mg. of culture of B III., and died 39 days afterwards.

Lungs.

The tissue shows a generally distributed, irregular thickening of the alveolar walls, and contains many miliary tubercles in an early stage of formation. No fibrin is present. The tubercles are moderately rich in bacilli, and bacilli also occur, here and there, in the rest of the tissue.

Liver.

Miliary tubercles are abundant. They are in an early stage of formation, and contain fairly numerous bacilli.

Kidneys.

No lesions or bacilli have been found.

Iliac Gland.

The gland shows advanced caseation, and contains dense masses of bacilli.

SUMMARY.

Acute, disseminated tuberculosis.

VIRUS.—B III.

Animal Inoculated.—Cat 36.

Cat 36 (kitten) received an intraperitoneal inoculation with 1 mg. of culture of B III., and died 17 days afterwards.

Lungs.

Apart from some small patches of alveolar thickening, which are marked by an infiltration of leucocytes, the tissue shows no noteworthy histological change. Bacilli are found everywhere within the alveolar walls, and are very numerous in the infiltrated foci.

Liver.

The tissue is riddled with miliary tubercles. Many of them are highly necrotic; in the less advanced, multinuclear leucocytes and their remains are abundant. Tubercle bacilli are abundant in all these foci; they also occur plentifully in the rest of the tissue, their presence being generally associated with some slight necrotic change and a determination of multinuclear leucocytes.

Kidneys.

There are no histological lesions in the specimen examined, but a few groups of bacilli have been found.

Ileo-Colic Gland.

The gland shows many caseous areas in which bacilli are abundant.

SUMMARY.

The lungs show only a slight amount of tissue change but contain large numbers of bacilli. The liver is very acutely infected.

VIRUS.—B IX.
Animal Fed.—Cat 42.

Cat 42 (kitten) was fed with 1 mg. of culture of B IX., and was killed 9 days afterwards.

Lungs.

In the section examined there are two small tubercles, which are densely infiltrated with leucocytes and lymphocytes. In one of these a tubercle bacillus is present.

Mesenteric Gland.

In the gland examined neither tubercles nor tubercle bacilli have been found.

SUMMARY.

Slight infection of the lungs.

VIRUS.—B IX.
Animal Fed.—Cat 48.

Cat 48 (kitten) was fed with 1 mg. of culture of B IX., and died 47 days afterwards.

Lungs.

The alveolar walls show a great deal of irregular thickening; and here and there patches, the size of several alveoli, are completely consolidated; they are not caseous. The alveolar walls, and particularly the larger consolidated areas, contain large numbers of multinuclear leucocytes. Tubercle bacilli are present in these areas, but only occur in very scanty numbers.

Liver.

Several minute tubercles are present. They are filled up with lymphocytes and leucocytes, and, with the exception of one which is very slightly caseous. show no evidence of breaking down. Giant cells are occasionally found. Only one tubercle bacillus has been discovered.

Kidneys.

No tubercles or tubercle bacilli have been found.

Mesenteric Gland.

The gland is extensively caseous and contains large numbers of bacilli.

SUMMARY,

The lung alveoli, which show irregular thickening of their walls and are occasionally consolidated, contain a few bacilli. The liver contains many small, obviously retrogressive tubercles. The mesenteric glands are caseous and contain numerous bacilli.

VIRUS.—B IX.
Animal Fed.—Cat 50.

Cat 50 (kitten) was fed with 1 mg. of culture of B IX., and died 74 days afterwards.

Lungs.

The general appearance of the lungs resembles that found in Cat 48, but the consolidated areas are more numerous, and some of them show a little evidence of caseation. There is a sprinkling of plasma cells at the periphery of these areas. No fibrin is present. Tubercle bacilli are found scattered in small numbers in all the lesions. They are therefore distinctly more numerous than in the lungs of Cat 48.

Liver.

A few minute foci of doubtful nature, and not containing any tubercle bacilli, have been found.

Kidneys.

No tubercles or tubercle bacilli have been found.

Mesenteric Gland.

Many caseous foci are present. In these, tubercle bacilli are extremely numerous.

SUMMARY.

Moderately extensive tuberculosis of the lungs has been produced.

VIRUS.—B IX.
Animal Fed.—Cat 52.

Cat 52 (kitten) was fed with 1 mg. of culture of B IX. and died 121 days afterwards.

Lungs.

The specimen shows more advanced disease than the lungs of either of the two preceding animals. The tissue is nearly completely solidified, and the lesions have the patchy distribution typical of tubercular pneumonia. The solidified areas are just commencing to caseate. Plasma cells are distributed in moderately large numbers at the periphery of these areas. Tubercle bacilli are present in moderately large numbers.

Liver.

There are several very minute tubercles. These are not caseous but are filled with leucocytes and lymphocytes. In a few of these nodules one or two tubercle bacilli are present.

Kidneys.

No tubercles or tubercle bacilli have been found.

Bronchial Gland.

The gland examined shows numerous foci of early caseation. In all these, bacilli are fairly numerous.

SUMMARY.

Severe tuberculosis of the lungs. Minute, retrogressive lesions in the liver.

VIRUS.—B XVIII.

Animal Inoculated.—Cat 66.

Cat 66 was inoculated subcutaneously with 1 mg. of culture of B XVIII., and died 70 days afterwards.

Lungs.

The tissue is in a condition of incomplete consolidation, and contains numerous areas which are commencing to caseate. At the periphery of these areas there is a well marked layer of plasma cells. No fibrin is present. Tubercle bacilli are somewhat scanty.

Liver.

Fairly numerous minute tubercles are present, but no bacilli have been found in them.

Kidneys.

The specimen contains several large tubercles, both in the cortex and in the medulla. The centre of most of these is completely caseous. All the tubercles have a very dense cellular periphery, which consists mainly of fibro blasts and a very abundant infiltration of plasma cells. The tubercles contain numerous bacilli. The bacilli have grown into small colonies and are evidently multiplying freely.

Iliac Gland.

Many caseating foci are present which contain numerous bacilli.

Summary.

Extensive disease of the lungs and kidneys. The bacilli are not numerous in the lungs, but are multiplying freely in the kidneys.

VIRUS.—B XXVIII.

Animal Inoculated.—Cat 76.

Cat 76 was inoculated subcutaneously with ·1 mg. of culture of B XXVIII. and died 22 days afterwards.

Lungs.

The lungs are highly congested and here and there a few alveoli are consolidated and infiltrated with leucocytes. No definite tubercles have been found. Tubercle bacilli are numerous and are disseminated throughout every part of the tissue. The bacilli vary in length from 1·5 to 4·5μ, are often curved, and are generally stained uniformly.

Liver.

Very numerous, minute, necrotic foci, exhibiting no definite structure, are present. Both in these foci and in the rest of the tissue tubercle bacilli are present, everywhere, in large numbers. They are very frequent in the blood-capillaries.

Kidneys.

No tubercles have been found, but bacilli are present in small numbers within glomeruli, in the interstitial tissue, and within blood-capillaries.

Sternal Gland.

Large, ramifying, necrotic areas are present. In these, tubercle bacilli are present in enormous numbers. They are also found distributed in small numbers throughout the rest of the gland.

Summary.

The lungs, liver, and lymphatic glands are infiltrated with enormous numbers of tubercle bacilli. Bacilli are also present in the blood-stream. No typical tubercles have been found.

VIRUS.—B XXVIII.

Animal Inoculated.—Cat 84.

Cat 84 (kitten) was inoculated subcutaneously with 1 mg. of culture of B XXVIII. and died 21 days afterwards.

Lungs.

The tissue is highly congested and many of the alveoli are consolidated, but there are no definite tubercles. No fibrin is present. Numerous bacilli are present in every part of the tissue, and they are particularly abundant in the consolidated areas. The bacilli vary in length from 2 to 4·5μ, are often curved, and are generally stained uniformly.

Liver.

Similar to the liver of Cat 76. No fibrin is present.

Kidneys.

Similar to the kidneys of Cat 76, but bacilli more numerous. No fibrin present.

Sternal Gland.

Almost completely necrotic, and swarming with colonies of tubercle bacilli.

Summary.

Similar to Cat 76.

B. LESIONS PRODUCED BY HUMAN BACILLI.

None received for examination.

C. SUMMARY.

These investigations support the experimental fact that infection can be readily produced in the cat both by feeding and by inoculation. The bacilli employed have been, in every case on which I have reported, of bovine origin. In some cases typical tubercles have been produced; in others the tissue has been swarming with bacilli, but no typical tubercles have been formed.

EXPERIMENTS ON RATS.

A. LESIONS PRODUCED BY BOVINE BACILLI.

VIRUS.—B I.

Animal Inoculated.—Rat 47 (B).*

Rat 47 (B) was inoculated intraperitoneally with 15 mg. of culture from Pig 16 (B I.) and died 54 days afterwards.

Lungs.

Histological Changes.—The lung is highly œdematous and semi-solid. Both the alveolar and the bronchial epithelium have desquamated. The general condition is typical of a severe catarrhal affection, and is not representative of a tubercular process.

Distribution and Characters of Bacilli.—Tubercle bacilli are plentiful and often form conspicuous colonies, but they have not overrun the specimen. They are generally intracellular, occur abundantly within giant cells, which are large and numerous, and exhibit a marked tendency to grow as a continuous layer completely surrounding cell nuclei.

The bacilli are from 2 to 5μ long. The great majority are curved. Small, globular swellings, irregular thickenings and regular beading are all very commonly found. Uniformly stained forms are the exception.

Liver.

Histological Changes.—There is a slight degree of infiltration, especially noticeable at the periphery of veins. There are no tubercles or caseous areas, and no definitely formed giant cells. The tissue is apparently somewhat affected by post-mortem changes, as the more delicate staining methods are unsuccessful. No fibrin is demonstrable.

Distribution and Characters of Bacilli.—Bacilli are numerous throughout the tissue and are generally intracellular, but they have not grown together into solid masses.

The bacilli are similar, morphologically, to those present in the lung.

Kidneys.

Histological Changes.—General infiltration, both in the cortex and the medulla ; slight, on the whole, but with a few denser aggregations. No other evident lesions. Tissue does not react to delicate stains.

Distribution and Characters of Bacilli.—Tubercle bacilli occur in many small groups, both in the cortex and the medulla, but are not universally distributed. Some are found within glomeruli, but they more usually occur within the interstitial tissue. They are usually intracellular, and appear occasionally to be within renal cells.

The bacilli have the same morphological appearance as those found in the liver and lung.

SUMMARY.

There is a general invasion with tubercle bacilli, which have greatly increased in numbers. The bacilli are for the most part intracellular ; they have produced relatively little tissue destruction and no definite tubercles.

VIRUS.—B IV.

Animal Inoculated.—Rat 42 (B).

Rat 42 (B) was inoculated intraperitoneally with 1 mg. of culture of B IV., and was killed 268 days afterwards.

Lungs.

The alveolar walls are greatly thickened and in many places groups of alveoli are consolidated. There is no caseation and no tubercle formation. Giant cells occur rather frequently. The infiltration of the alveoli consists of leucocytes, lymphocytes, and cells which react to Pappenheim's stain for plasma cells. These last are numerous and form a conspicuous feature in the specimens. No fibrin is present. Bacilli are numerous throughout the tissue ; they are usually intracellular and have frequently grown into groups. They vary in length from 1·5 to 4·5μ, the longer being curved.

Liver.

The tissue contains many small foci, consisting of no more than an aggregation of a few leucocytes and lymphocytes. In some of these lesions tubercle bacilli are present, but they are not numerous.

Kidneys.

No lesions or tubercle bacilli have been found.

Mediastinal Gland.

The gland shows no marked histological change, but contains bacilli in scanty numbers.

SUMMARY.

The bacilli have reached the lungs, and there many have survived and multiplied.

VIRUS.—B VI.

Animal Inoculated.—Rat 37 (B).

Rat 37 (B) was inoculated subcutaneously with 1 mg. of culture of B VI., and was killed 205 days afterwards.

Specimens of the lungs, liver, kidneys, and an inguinal gland of this animal have been microscoped. No tubercular lesions have been found and, with the exception of the lung, in which four fragmentary tubercle bacilli have been detected, no tubercle bacilli are demonstrable.

The case is recorded as an instance of the resistive powers of the rat against virulent tubercle bacilli when administered subcutaneously in a dose which, relatively to this animal, must be considered small.

SUMMARY.

Hardly any trace of infection.

* B.=Infected with bovine bacilli.

VIRUS. B VII.

Animal Inoculated.—Rat 48 (B).

Rat 48 (B) was inoculated subcutaneously with 10 mg. of culture of B VII., and died 49 days afterwards.

Lungs.

Histological Changes.—There is much catarrhal change, but nothing suggestive of a tubercular process.

Presence of Bacilli.—After searching through two specimens a few tubercle bacilli were found. They occurred within desquamated epithelial cells.

Inguinal Gland.

Histological Changes.—Many small, circular foci from which the lymphoid cells have disappeared. Staining reactions unsatisfactory.

Distribution and Characters of Bacilli.—The areas mentioned above are all filled with bacilli. Bacilli also occur in small groups in other situations not marked by any histological change. A few bacilli are also found amongst the areolar and fatty tissue surrounding the gland.

The bacilli are almost all short and straight, their length rarely exceeding 2μ, and very frequently being 1μ or less. Except those which are evidently becoming broken up, they are uniformly stained.

SUMMARY.

The bacilli have multiplied abundantly in the inguinal gland, but extremely few are present in the lungs.

VIRUS.—B VIII.

Animal Inocluated.—Rat 61 (B).

Rat 61 (B) was inoculated intraperitoneally with 10 mg. of culture of B VIII., and died 9 days afterwards.

Unfortunately the tissues have proved to be too necrotic to stain well, but a few details are worth recording, as illustrating the distribution of bacilli 9 days after inoculation.

The lungs, which are in a catarrhal condition, contain many small groups of bacilli, amongst which multinuclear leucocytes and their remains are frequently found. The bacilli are from 1·5 to 3μ in length, generally straight and frequently beaded.

Throughout the liver bacilli are abundantly distributed in small groups, both in the interstitial tissue and amongst the liver cells. Morphologically, as compared with those found in the lungs, the bacilli are rather longer and more frequently curved.

The renal tissue has not been invaded by bacilli, but masses of these organisms are found adherent to some parts of the capsule. They are noticeably shorter than those either in the lungs or liver.

Comparing these specimens with those of other rats which have been examined, there is no histological evidence that death was due to tuberculosis.

SUMMARY.

The lungs contain many small groups of bacilli, 9 days after intraperitoneal inoculation. Throughout the liver, bacilli are abundant.

VIRUS.—B XII.

Animal Inoculated.—Rat 55 (B).

Rat 55 (B) was inoculated subcutaneously with 10 mg. of culture of B XII., and died 62 days afterwards.

Lungs.

There is some catarrhal change, and irregular thickening of the alveolar walls. Giant cells occur frequently. There are no tubercles. The cells which have infiltrated the alveolar walls are lymphocytes, leucocytes, and plasma cells. None of the giant cells react to Pappenheim's differential stain for plasma cells. Tubercle bacilli are present throughout the tissue ; they generally occur in small groups, and are often fragmentary. All the giant cells contain bacilli. The bacilli vary in length from 1·5 to 4.5μ, the longer being curved.

Liver.

There are many small foci, which contain an aggregation of lymphocytes and leucocytes. Small groups of bacilli, many of them fragmentary, are present in these areas.

Kidneys.

Tubercle bacilli are found here and there in the interstitial tissue, but there are no definite histological lesions.

SUMMARY.

There is a general dissemination of bacilli, but they have not produced any tissue destruction. Many of the bacilli are disintegrated.

VIRUS.—B XVI.

Animal Inoculated.—Rat 73 (B).

Rat 73 (B) received an intraperitoneal inoculation with 10 mg. of culture of B XVI., and died 40 days afterwards.

Lungs.

The tissue is in a condition of nearly complete consolidation. Beneath the pleura there is a necrotic focus which is filled with masses of broken down leucocytes and is surrounded by fibroblasts. Internal to the fibroblasts there is a zone of cells of epithelial type. The rest of the tissue is not necrotic, but contains very numerous giant cells. Some fibrin is present in the necrotic nodule. In the centre of the necrotic nodule tubercle bacilli are scanty, but they are abundant amongst the epithelial cells within the periphery of the nodule. In the rest of the tissue tubercle bacilli are present in enormous numbers, forming conspicuous red colonies in all parts of the section. The bacilli vary in length from 2 to $4{\cdot}5\mu$, straight and curved forms being about equally numerous.

Liver.

No tubercles or other conspicuous lesions are present, but the tissue is overrun with bacilli, which have grown into small clusters in all parts of the specimen. The bacilli have a marked tendency to grow in the form of a wreath surrounding a cell nucleus.

Kidneys.

There are no definite lesions, but minute groups and colonies of bacilli occur abundantly in all parts of the specimen.

Bronchial Gland.

Many colonies of bacilli are present, but there is no appreciable tissue change. The majority of the bacilli are extremely small.

SUMMARY.

The bacilli have been disseminated all over the body and have multiplied enormously, but, except in the lungs, there is very little tissue change.

B. LESIONS PRODUCED BY HUMAN BACILLI.

VIRUS.—H. 13. A.D.

Animal Inoculated.—Rat 15 (H).*

Rat 15 (H) received an intraperitoneal inoculation with an emulsion of the tissues of Calf 301, estimated to contain 50,000,000 bacilli, and was killed 167 days afterwards.

Lungs.

Histological Changes.—The solidification of the tissue is nearly complete; but empty, distended alveoli are found here and there, sometimes singly and sometimes in small groups. There is no necrosis; all the solidified tissue is packed with cells. There are no giant cells.

Multinuclear leucocytes are scarce, and only a few of those present will take a granular, eosinophil stain. The ordinary small lymphocyte, with a round, deeply staining nucleus, is more abundant, but is not the predominant type of cell present. Plasma cells are infrequent and stain faintly, but they are to be found in small groups, and sometimes form a ring encircling a blood-vessel. The substance of the consolidated tissue is composed mainly of cells with very imperfectly defined protoplasmic outlines, the general appearance in many situations being that of a mass of nuclei embedded in a protoplasmic matrix. Many of these nuclei are elongated or oval, and are apparently of endothelial or connective tissue origin. A very considerable number are circular in outline, pale, and about twice the diameter of the nucleus of ordinary small lymphocytes; possibly many of these actually are the swollen and degenerate nuclei of small lymphocytes. There is no increase of adult fibrous tissue. The alveolar epithelium has disappeared, or at least is unrecognisable as such.

Some of the capillary channels have been obliterated; but many are still patent, though compressed, and contain red corpuscles.

No fibrin is present.

There is a diminution in the amount of elastic tissue.

Distribution and Characters of Bacilli.—The number of bacilli is so enormous that the entire area of the specimen appears, under a low power, like a section of a culture speckled with blue points which represent the cell nuclei. Under a higher magnification the bacilli are found to be distributed in equal abundance almost everywhere, the only situation which appears free from them being the lumen of intact blood-vessels. They have a marked tendency to grow in clusters round cell nuclei.

The majority of the bacilli are from 2 to 3μ in length, straight, or only slightly curved, and uniformly stained. Longer forms, about 4μ, with a well-marked curve are not uncommon.

Liver.

Histological Changes.—Very little change is observable with a low power. There is a little infiltration within Glisson's capsule and also adjoining some of the hepatic veins. There are no recognisable tubercles and no giant cells.

On careful examination with a higher power many small irregularities in the interlobular liver tissue may be noted. Many of these would be overlooked in specimens not stained for bacilli; they are therefore described in their relation to the bacilli. Oxyphil leucocytes are infrequent, and so are plasma cells; neither form marked aggregations.

Distribution and Characters of Bacilli.—In the interstitial tissue within Glisson's capsules and in the infiltration noted elsewhere, bacilli are fairly numerous and have a tendency to cling to cells. Bacilli are also found scattered in small numbers throughout the intralobular tissue. These bacilli are present in minute foci, which are composed of liver cells more or less disintegrated, cells with flattened or oval nuclei which are suggestive of endothelial cells, and occasionally a few small lymphocytes. Sometimes, apparently when the section happens to pass through the centre of one of these minute spherical foci, the interrelationship of these cells is rather interesting. They are arranged in a concentric fashion, bearing a slight resemblance to a cell-nest, with the somewhat compressed liver cells in the centre, and the cells with flattened elongated nuclei at the periphery. It is often very difficult to say categorically that a bacillus is actually within a cell and not simply adhering to it, but in this liver it appears reasonably certain in many instances that bacilli, singly and in groups, are actually within liver cells. The nuclei of these bacilli-containing cells sometimes take a slightly deeper chromatin stain than the surrounding normal liver cells, and do not always preserve a completely circular contour. Bacilli are not found within the blood-vessels.

Morphologically, the bacilli resemble, on the whole, those found in the lungs, the only difference being that some of the longer intracellular forms shows a well-marked beading.

SUMMARY.

The lungs are to a large extent consolidated, but are not necrotic and present no appearance suggestive, histologically, of tuberculosis. They are, however, packed with tubercle bacilli, which form an almost solid mass throughout the tissue. Bacilli are also numerous in the liver, though there is very little histological change.

VIRUS.—H. 16. J.H.

Animal Inoculated.—Rat 28 (H).

Rat 28 (H) received an intraperitoneal inoculation with 100 mg. of culture from Guinea-pig 609, which had been inoculated with the original material. This culture was ascertained to be of low virulence for bovines. Rat 28 died 172 days after inoculation.

Lungs.

Histological Changes.—The general condition of the lungs is one of incomplete consolidation. Complete obliteration of alveolar spaces, great distension and dense infiltration of the alveolar walls, partial or complete

*H = Infected with human bacilli.

occlusion of alveoli, with desquamated cells or coagulated deposit, and great distension of completely empty alveoli are all found side by side in the same section. There is no necrosis. There are some giant cells, but there are no areas which may be described as tubercles.

In the situations where the morbid changes are those of an ordinary catarrhal process, there are masses of broken-down leucocytes, but in the rest of the tissue these cells, though always present, are relatively infrequent. Small lymphocytes occur in small groups at the periphery of consolidated patches, and individual cells of this class are to be found everywhere; these cells, however, do not form the predominant feature of the lesions: no area can be described as overrun with them. Plasma cells are present, stain well, and are of characteristic appearance, exhibiting every transition from the cell indistinguishable from a large lymphocyte to the narrow, compressed cell of rectangular outline. They occur in groups at the periphery of consolidated patches, bronchioles, and blood-vessels, and are found occurring singly in all situations. An interesting feature of this lung is the increase of the alveolar epithelium. A great many alveoli are filled with alveolar epithelial cells which have partially reverted to the embryonic type.

There is some loss of elastic tissue.

Distribution and Characters of Bacilli.—Compared with Rat 15 H, where the bacilli were so abundant everywhere as to largely obscure even the cell nuclei, the bacilli here are much less numerous. The low power view shows a liberal sprinkling of red specks scattered everywhere, but rarely so densely aggregated as to obscure the tissue. In the catarrhal patches they are rather less plentiful than in the completely consolidated patches. They are found adherent to, or within, every type of cell, and exhibit a marked tendency to grow as small balls, each of which has a cell-nucleus for its core.

The majority of the bacilli are curved, and from 2·5 to 4·5μ in length; forms as long as 6 or 7μ are not infrequent. A small proportion of the bacilli are beaded.

Liver.

Histological Changes.—There is a well marked infiltration within Glisson's capsules and surrounding all the vessels, both portal and hepatic. There is also a slight degree of infiltration amongst the liver cells. There are no tubercles, but there are occasional formations suggestive of imperfectly formed giant cells.

The infiltration consists chiefly of small lymphocytes and plasma cells, the former being much more numerous than the latter. Multinuclear leucocytes occur here and there, but rather infrequently, and never form aggregations. In the infiltrated areas, particularly within Glisson's capsules, are generally found a few large cells filled with coarse basophil granules.

Distribution and Characters of Bacilli.—Bacilli are sufficiently numerous to be just visible with a low power within Glisson's capsules. Small colonies of them, each colony being about the size of a liver cell, are also seen in various parts of the tissue. The great majority of the bacilli are intracellular. They show no relationship to the basophil cells, and are not contained within them. they are frequently found within parenchymatous liver cells. Small foci in the liver parenchyma similar to those described as found in the liver of Rat 15 (H) are of frequent occurrence.

The bacilli are long and curved, like those found in the lung, but are rather more frequently beaded.

Kidneys.

Histological Changes.—A slight degree of interstitial infiltration, both in the cortex and the medulla, but never forming dense aggregations. No necrotic change, no tubercles, and no giant cells.

The infiltration consists mainly of small lymphocytes. An occasional plasma cell is found. There is a slight increase of fibrous tissue.

Distribution and Characters of Bacilli.—With a low power aggregations of bacilli are just recognisable in some parts of the cortex, both in the interstitial tissue, and in the glomeruli; they are more clearly recognisable in the medulla, where the patches are larger and the bacilli more densely packed together. The bacilli are not universally distributed; several parts of the section contain none. The general process seems to be that bacilli which have been carried into the circulation have attached themselves here and there to cells, and then proceeded to grow round the cell nuclei. There is a remarkable absence of tissue destruction. Many glomeruli contain no bacilli, but those which do contain any are generally packed with them, without any extension of the organisms beyond the glomerulus. Both in the cortex and the medulla, the bacilli, though more frequent in the interstitial tissue than amongst the renal cells, are not uncommonly found attached to or growing round the nuclei of renal cells. In the medulla the absence of tissue destruction is even more striking than in the cortex. Masses of bacilli are found glued together as closely as in lumps of culture, and yet have produced hardly any irritant, and no destructive effect upon the adjacent tissue.

Morphologically, the bacilli resemble those found in the liver.

Lymphatic Glands.

Three small lymphatic glands, taken from the abdominal cavity, have been microscoped.

Histological Changes.—In one of them a good deal of the lymphoid tissue has disappeared; the recticular framework of the gland is not well defined and shows an excess of fibroblasts. Large lymphocytes are demonstrable at the periphery of the gland and some are also seen in the interior, but a great many of them have disappeared. The other two glands appear histologically normal. They are crowded with large lymphocytes which, as is the case with those observed in the first gland, stain deeply by Pappenheim's method. All three glands contain large basophil cells.

Distribution and Characters of Bacilli.—The first of the three glands mentioned is filled with masses of bacilli which are equal in abundance to those found in the lungs of Rat 15 (H). In the other two glands bacilli are present, but, for the rat, are very scanty.

The bacilli resemble morphologically those found in the liver.

Summary.

There is a general dissemination of tubercle bacilli, and the lungs are partially consolidated.

VIRUS.—H 16. J.H.

Animal Inoculated.—Rat 30 (H).

Rat 30 (H) received an intraperitoneal inoculation with 30 mg. of culture isolated from Calf 423A, and died 258 days afterwards. This culture was found to be of high virulence for bovines.

Lungs.

The tissue is congested and partially consolidated. The alveolar walls are greatly thickened, and many of the alveoli are occluded. There is no necrosis, nor is there any formation of tubercles. Giant cells are present. Tubercle bacilli occur throughout the tissue, but, for the rat, in relatively scanty numbers. They often form small groups but are never densely aggregated together, and do not form colonies visible under a low power. The bacilli vary in length from 1·5 to 4·5μ; straight and curved forms are equally numerous.

Liver.

There is very little histological change, but here and there a small collection of cells, chiefly lymphocytes is noticeable. Both in these situations and in many other parts of the tissue, when there is no histological abnormality, tubercle bacilli are present. Their number

is scanty, though they are often found in small groups. Many of the bacilli are fragmentary.

Kidneys.

There is no histological change. Tubercle bacilli are present but scanty.

Mesenteric Gland.

There are a great many small foci which show slight necrotic change. In these, bacilli are fairly numerous, but they are loosely distributed ; they are never packed closely together. A considerable number of the bacilli are fragmentary.

SUMMARY.

Bacilli are distributed throughout the body, but in relatively small numbers, and there is evidence that they are not able to multiply actively.

VIRUS.—H 16. J.H.

Animal Inoculated.—Rat 36 (H).

Rat 36 (H) received an intraperitoneal inoculation with an emulsion of the tissues of Rat 28 (H), and died 135 days afterwards.

Lungs.

The tissue is highly congested and almost completely solidified. There is no necrosis, and no formation of tubercles. The tissue is swarming with bacilli, which form red points and groups visible with a low power all over the section. Groups of bacilli are often found closely wrapped round cell nuclei. Some of the bacilli are broken up into fragments, but a large number of them are long and curved, measuring from 3·5 to 6μ.

Liver.

Small groups of bacilli occur in all parts of the tissue. These groups are scantier and much smaller than those found in the lungs, and the bacilli which form them are not closely packed together. In some of the foci there is partial destruction of the liver cells, and a small aggregation of lymphocytes, with an occasional leucocyte ; but in many situations where bacilli are present there is no histological change.

Kidneys.

There is no noteworthy histological change, with the exception of one small focus where there is an aggregation of leucocytes. This focus contains several solid masses of bacilli. Bacilli are also present in many other situations, forming clumps and wreath-like masses.

SUMMARY.

The lungs are nearly solid and contain enormous numbers of bacilli. Bacilli are also growing freely in the kidneys but have produced very little tissue change.

VIRUS.—H 17. Sp. B.

Animal Inoculated.—Rat 34 (H.)

Rat 34 (H) received an intraperitoneal inoculation with 80 mg. of a culture derived from Calf 339, and died 97 days afterwards.

Lungs.

The tissue is semi-solid, but shows no necrosis or tubercle formation. Giant cells are occasionally found. Tubercle bacilli are very abundant and are distributed with remarkable uniformity all over the tissue. They are everywhere growing into small clusters, and are very frequently intracellular. Long and short forms are about equally numerous, the length varying from 2 to 6μ.

Liver.

The tissue shows a general infiltration with lymphocytes and leucocytes. Tubercle bacilli are abundant and are uniformly disseminated in small groups throughout the substance of the organ.

Kidneys.

No histological lesions have been found. Tubercle bacilli are disseminated in small numbers throughout the tissue, and occasionally have grown into minute colonies.

Mesenteric Gland.

Bacilli are numerous, but have produced very little tissue change.

SUMMARY.

Bacilli are abundant throughout the body, but have produced relatively little tissue change.

VIRUS.—H 19. S.W.

Animal Inoculated.—Rat 13 (H.)

Rat 13 (H) received an intraperitoneal inoculation with over 5,000,000 bacilli from the tissues of Calf 271, and was killed 180 days afterwards.

Lungs.

Histological Changes.—Within the lung substance there are many small irregularly outlined areas of consolidated tissue, which, as seen under a low power, appear to consist chiefly of small round cells. These areas are not demarcated from the rest of the tissue ; they are not caseous, but contain several giant cells. More or less continuous with these areas, the alveolar walls are thickened and infiltrated with cells of the small round type. The rest of the lung tissue shows irregular patches of congestion and some desquamation of epithelial cells. Both in the infiltrated and in the merely congested areas giant cells are occasionally found.

Some of the bronchioles are filled with multinuclear leucocytes, possessing finely granular oxyphil protoplasm. These cells are also fairly frequent in the congested areas ; they are only rarely met with in the areas of dense cellular infiltration. The preponderating element in the thickened and infiltrated alveolar walls, and also in the larger consolidated areas consists of small lymphocytes. Associated with these cells is a much smaller number of larger cells, which react to Pappenheim's stain, and are indistinguishable from ordinary large lymphocytes. Cells with the typical varieties of shape, and the typical staining reaction of plasma cells are also to be found. They are most numerous at or near the periphery of bronchioles. In the consolidated areas and to a large extent obscured by the lymphocytes is a framework of cells with large, pale, oval or elongated nuclei, and imperfectly defined protoplasm. These, or at least many of them, are probably derived from the capillary walls. Except in the smaller and earlier foci, there is little or no evidence of the survival of alveolar epithelium.

Distributions and Characters of Bacilli.—Tubercle bacilli occur throughout the tissue. Even in situations which are histologically normal, or show no change beyond capillary engorgement, some bacilli, generally within alveolar epithelial cells, can be found in nearly every

microscopic field. In the more definitely affected areas they are more numerous and frequently occur in small clusters, each of which consists of about ten to fifty bacilli. The bacilli have a marked tendency to occur within cells, particularly epithelial cells, endothelial cells, and fibroblasts, and seem to have multiplied in these situations. I have not noticed any within multinuclear leucocytes.

Some of the intracellular bacilli are broken up into short fragments. With the exception of these forms, the bacilli vary in length from 1·5 to 2·5 or 3μ; they are uniformly stained and either straight or slightly curved.

Liver and Kidneys.

Sections of these organs have been examined, but no tubercle bacilli have been found.

SUMMARY.

The lungs are partially consolidated and contain numerous bacili. No bacilli have been found in the liver or kidneys.

VIRUS.—H 19. S.W.

Animal Inoculated.—Rat 14 (H).

Rat 14 (H) was inoculated subcutaneously with the same dose of the same material as Rat 13 (H), and was killed 180 days afterwards.

Lungs.

Histological Changes.—The blood capillaries are engorged with red corpuscles, and in some places the alveolar walls are thickened and contain many small round cells. In one small patch close to the surface this determination of small cells is particularly marked, and there is a desquamation of alveolar epithelium.

In the situation last mentioned, multinuclear leucocytes are very numerous; a few plasma cells are demonstrable but faintly stained; small lymphocytes are frequent, but not more numerous than the leucocytes; the protoplasm of many of the epithelial and endothelial cells is imperfectly defined; and there is a slight tendency to cell fusions. The rest of the tissue contains multinuclear leucocytes in much smaller numbers, and does not exhibit any plasma cells, either at the periphery of bronchioles and vessels or elsewhere.

Distribution and Characters of Bacilli.—Several tubercle bacilli are found in the partially consolidated patch beneath the pleural surface which has been described above, but none elsewhere. Many of the bacilli are broken up. Those which are not are from 2 to 3μ in length, uniformly stained and slightly curved. The tubercle bacilli which are morphologically normal are very scanty and never form groups.

Liver and Kidneys.

No lesions or tubercle bacilli are demonstrable.

Inguinal Gland.

Histological Changes.—There are many small, pale areas consisting of cells with large, pale, oval or rounded nuclei and ill-defined protoplasm. These cells appear to be mainly endothelial cells covering the reticular framework of the gland. Multinuclear oxyphil leucocytes are distributed throughout the gland, but are no more numerous within or near these areas than elsewhere. A few of the leucocytes are of the coarsely granular type.

Distribution and Characters of Bacilli.—In all the pale areas referred to bacilli are scattered in fairly large numbers. They are all short and the great majority of them are obviously fragmentary. They never form clumps. Single bacilli also occur here and there in the rest of the gland, apparently attached to endothelial cells or fibrous tissue corpuscles. As in the lungs, the bacilli present were probably not in an active condition.

SUMMARY.

The lungs show much less evidence of tubercular infection in this animal, inoculated subcutaneously, than in the companion animal which was inoculated intraperitoneally.

VIRUS.—H 20. F.L.

Animal Inoculated.—Rat 33 (H).

Rat 33 (H) was inoculated intraperitoneally with 70 mg. of culture of H. 20, and died 52 days afterwards.

Lungs.

Histological Changes.—There are irregular, semi-confluent areas of consolidation and, in the surrounding tissues, much infiltration and distension of the alveolar walls. Giant cells are fairly numerous, but there are no tubercles and there is no necrosis.

Multinuclear leucocytes are scanty. A few of the leucocytes present are of the coarsely granular type. Small lymphocytes are much more numerous; they are distributed everywhere, but never form dense aggregations. Cells with the staining reaction of plasma cells and indistinguishable in appearance from large lymphocytes are present in small groups in various parts of the tissue and stain well. Plasma cells of the rectangular and flattened type are not found. There is both desquamation and new growth of alveolar epithelial cells. Many of the giant cells appear to be formed by an aggregation of small lymphocytes round or at one end of a swollen and necrotic epithelial cell.

There is no new formation of adult fibrous tissue

There is some diminution in the amount of elastic tissue.

Fibrin is absent.

Distribution and Characters of Bacilli.—Small rings and clusters of bacilli are visible with a low power in nearly every field, but are generally some distance apart. With a higher magnification, bacilli are found to be freely distributed everywhere. Many of the larger clusters of bacilli, *i.e.*, those which are large enough to be visible with a low power, are found within the protoplasm and enveloping the nuclei of giant cells. In the rest of the tissue the bacilli exhibit the same general relationship to cells as those described in previous specimens, the only difference being that more isolated bacilli are found and the number of cell nuclei which are surrounded with bacilli is fewer. It is important to bear in mind that the duration of this experiment was much shorter than in the case of the previous animals.

The bacilli vary in length from about 2 to 4 or 4·5μ; the longer forms are curved and frequently beaded.

Liver.

Histological Changes.—The capsule is greatly thickened and appears in section as a cellular and vascular infiltration penetrating between broad bands of homogeneous, hyaline material. In the liver substance there is a well-marked infiltration resembling in general characters that described in the liver of Rat 28 (H), but more pronounced and more densely permeating the parenchymatous tissue.

Coarsely granular oxyphils are of rather frequent occurrence. In other respects the histological details are the same as in the liver of Rat 28 (H). No fibrin is present.

Distribution and Characters of Bacilli.—Bacilli occur plentifully everywhere and are nearly all intracellular. As contrasted with some of the specimens described above, where the experiment had run a longer course, the bacilli have not grown together into a solid mass closely wrapping round the nucleus, but are loosely arranged within the cell protoplasm. They often occur within the protoplasm of liver cells. Frequently bacilli are found amongst a group consisting of about half a dozen cells of different types closely blended together.

Some of these are liver cells and others are lymphocytes. As bacilli are not observed attached to the freely circulating lymphocytes, the probable sequence of events in the formation of these groups is that first the bacilli gain an entrance into the liver cells and multiply within them, then lymphocytes become adherent to these cells, and finally the two types of cells become entangled together and permeated with a mass of bacilli, the nuclei of the lymphocytes surviving longer than those of the liver cells.

The bacilli resemble morphologically those found in the lungs.

Kidneys.

Histological Changes.—Slight, generalised interstitial infiltration with some denser patches in the cortex. No necrosis, no tubercles, and no giant cells.

Histological details as in the kidney of Rat 28 (H,) with the exception that plasma cells are rather more numerous, and take a deep protoplasmic stain.

Distribution and Characters of Bacilli.—The distribution of the bacilli is identical with that found in the kidney of Rat 28 (H).

The bacilli vary in length from about 1·5 or 2μ to about 4μ. Many of the longer forms are curved. There is a tendency to beading in many both of the long and the short forms.

Lymphatic Glands.

Two pieces of lymphatic tissue taken from within the abdominal cavity and one inguinal gland have been microscoped.

Histological Changes.—These may be sufficiently described by comparing them with the glands of Rat 28 (H). The inguinal gland resembles the two glands from Rat 28 (H), which were described as histologically normal. The two abdominal glands resemble the gland from Rat 28 (H), in which marked histological changes were noted.

Distribution and Characters of Bacilli.—In the inguinal gland a few small groups of bacilli are found. The other two glands are packed with bacilli, the greater portion of the sections appearing under a low power as an aggregation of red balls and streaks. In one of the specimens containing abdominal lymphatic tissue a fragment of pancreas was accidentally included. The fibrous tissue surrounding the pancreas is swarming with bacilli, and an occasional bacillus is to be found amongst the interstitial pancreatic tissue, but the secretory cells are completely free from bacilli.

Many of the bacilli are fragmentary. Bacilli of all lengths from 1 to 5μ are found. Straight forms, even amongst the longer bacilli, are more frequent than curved. A good many of the bacilli are either regularly beaded or stained unequally.

SUMMARY.

The lungs are partially consolidated and contain very large numbers of bacilli. Bacilli are also distributed plentifully throughout the rest of the body.

VIRUS.—H 28. C.L.

Animal Inoculated.—Rat 24 (H).

Rat 24 (H) received an intraperitoneal inoculation of about 95 million bacilli obtained from the tissues of Calf 515, and died 340 days afterwards.

Lungs.

The tissue shows a good deal of catarrhal exudate and is partially consolidated. Tubercle bacilli are not numerous, but occur in small groups throughout the specimen. Their length varies from 2 to 4μ.

Liver, Kidneys, and Suprarenal Gland.

Tubercle bacilli are present in scanty numbers.

SUMMARY.

Tubercle bacilli are present in small numbers throughout the body.

VIRUS.—H 28. C.L.

Animal Inoculated.—Rat 27 (H).

Rat 27 (H) was inoculated with an emulsion of tissues from Calf 489 and died 343 days afterwards. The inoculation was found at the post-mortem examination to have been partly subcutaneous and partly intraperitoneal.

Lungs.

Tubercle bacilli are more numerous than in the lungs of Rat 24 (H) and occur in larger groups. Their length varies from 2 to 4μ.

Liver and Kidneys.

Tubercle bacilli are present in scanty numbers.

SUMMARY.

The lungs are more extensively invaded with tubercle bacilli than those of Rat 24 (H).

VIRUS.—H 28. C.L.

Animal Inoculated.—Rat 39 (H).

Rat 39 (H) received an intraperitoneal inoculation with 100 mg. of culture, and died 33 days afterwards.

Lungs.

The alveolar walls are greatly thickened and contain numerous giant cells. Tubercle bacilli are very numerous and have grown into small colonies in every part of the specimen. They vary in length from 2 to 5μ, straight and curved forms being equally numerous.

Liver.

The liver contains many giant cells, but no lesions which can be described as definite tubercles. Bacilli are very numerous and have grown into small colonies in all parts of the specimen.

Kidneys.

Bacilli are much less numerous than in the liver, but small groups and colonies are found in many parts of the specimen.

SUMMARY.

Tubercle bacilli are very abundant, but have caused no tissue destruction.

VIRUS.—H 29. M.F.

Animal Inoculated.—Rat 35 (H).

Rat 35 (H) received an intraperitoneal inoculation with 50 mg. of culture, and died 67 days afterwards.

Lungs.

The tissue is in a condition of nearly complete solidification. Giant cells are numerous. There is no necrosis. The tissue is swarming with tubercle bacilli, which have grown into colonies, closely set together, in all parts of the specimens. Their length varies from 2 to $4 \cdot 5\mu$.

Liver and Kidneys.

Lilke the lungs, these tissues are swarming with enormous numbers of bacilli. The kidneys show some cellular infiltration of the interstitial tissue, but both in the kidneys and in the liver the amount of tissue change is remarkably small considering the enormous numbers of bacilli present.

Summary.

There is an enormous multiplication of bacilli throughout the body.

VIRUS.—H 47. S.B.

Animal Inoculated.—Rat 37 (H).

Rat 37 (H) received an intraperitoneal inoculation with 100 mg. of culture and died 113 days afterwards.

Lungs.

The lungs are almost completely solidified. There are some small patches of commencing necrosis which are filled with multinuclear leucocytes and their remains. Giant cells are frequent. There are no lesions which present the histological structure of tubercles. Plasma cells form a conspicuous layer at the periphery of bronchioles and blood-vessels and are also found scattered in small numbers in the rest of the tissue. No fibrin is present. Tubercle bacilli are present in enormous numbers, forming everywhere dense colonies visible under a low power. Many of them are notably long, measuring from 4 to 5μ, or even 6μ.

Liver.

There is an increase of the interstitial tissue between some of the lobules, but no other notable tissue change. Bacilli are distributed everywhere in large numbers.

Kidneys.

There are no definite histological lesions, but small groups of tubercle bacilli are formed in many parts of the tissue.

Summary.

The lungs are to a large extent consolidated and show some necrotic foci. Tubercle bacilli are extremely abundant in the lungs ; they are somewhat less plentiful in the liver and are rather scanty in the kidneys.

VIRUS.—H 47. S.B.

Animal Inoculated.—Rat 38 (H).

Rat 38 (H) received an intraperitoneal inoculation with nearly 100 mg. of culture and died 160 days afterwards.

Lungs.

The tissue is almost completely consolidated. Giant cells are numerous. There are no necrotic foci and there is no evidence of tubercle formation. Tubercle bacilli, though distributed throughout the tissue and forming numerous groups which are large enough to be visible under a low power, are not nearly so numerous as in the lungs of Rat 37 (H). As in the last-mentioned animal, many of the bacilli are long and curved.

Liver.

The liver shows a slight degree of infiltration with lymphocytes and leucocytes. Bacilli occur singly and in small groups. They are much less numerous than in the liver of Rat 37 (H).

Kidneys.

No lesions or tubercle bacilli have been found.

Summary.

The bacilli have multiplied much less abundantly than in the companion animal, Rat 37 (H).

VIRUS.—H 50. P.H.

Animal Inoculated.—Rat 40 (H).

Rat 40 (H) received an intraperitoneal inoculation with 74 mg. of culture and died 87 days afterwards.

Lungs.

The lungs are highly congested and in a condition of nearly complete consolidation. There is no necrosis or tubercle formation. Plasma cells form a conspicuous ring round the bronchioles and blood-vessels and are also distributed irregularly throughout the tissue. No fibrin is present. The tissue is very densely packed with tubercle bacilli, which form colonies, closely set together, in every part of the specimen. A large number of the bacilli are long and curved, their length varying from 2 to 6μ.

Liver.

A portion of the capsule is thickened and shows numerous fibroblasts and lymphocytes, together with some leucocytes. There is a slight infiltration of lymphocytes and leucocytes throughout the substance of the organ. Tubercle bacilli are very numerous, occurring in small groups and colonies throughout every part of the tissue.

Kidneys.

There is a slight increase of the interstitial tissue. Tubercle bacilli are numerous in all parts of the specimen.

Summary.

There is a general dissemination of tubercle bacilli. The bacilli have undergone particularly abundant multiplication in the lungs.

VIRUS.—H 54. C.W.

Animal Inoculated.—Rat 45 (H).

Rat 45 (H) received an intraperitoneal inoculation with 85 mg. of culture, and died 13 days afterwards. (Cause of death unknown.)

Lungs.

The alveolar walls are greatly thickened and the blood capillaries are engorged. In some of the alveolar walls there are groups of plasma cells. No fibrin is present. Small collections of bacilli are distributed at irregular but fairly close intervals. Many of these are identical with serum-grown bacilli and are obviously particles of the material inoculated. But in several places longer and more loosely distributed bacilli are present. It is evident, therefore, that the bacilli are commencing to multiply. The bacilli vary in length from 2 to 5μ.

Liver.

Large aggregations of bacilli are present beneath the capsule, and throughout the substance of the liver bacilli are freely distributed in large numbers. In many cases, particularly in places immediately beneath the capsule, the organisms are identical with serum-grown bacilli, but in the substance of the liver longer, curved forms preponderate. The deposits of bacilli are generally associated with small aggregations of leucocytes and lymphocytes and a swelling of the endothelial cells.

Kidneys.

No lesions or tubercle bacilli have been found.

SUMMARY.

Rapid dissemination of the bacilli has taken place in the liver. Bacilli are also present, but in much smaller numbers in the lungs. In both situations the organisms show evidence of multiplication.

MIXED HUMAN VIRUSES.

Animal Fed.—Rat 20 (H).

Rat 20 (H) died after being fed for 340 days with organs of tubercular guinea-pigs and with infected milk.

Lungs.

Histological Changes.—The lungs are somewhat congested, and there is some desquamation of epithelial cells. Some of these cells are found fused together and lying free in the alveoli. There are also to be found, lying free in the alveoli, cells which look like typical giant cells. It is also noteworthy that leucocytes and, more particularly, small lymphocytes, have a tendency to cling to the bodies of swollen epithelial or "dust" cells which, from the failure of their nuclei to take up any stain, may be regarded as moribund. The adherent lymphocytes or other cells retain their nuclear stain, and it is possible that the formation of some of the apparently typical giant cells may be accounted for in this way.

Distribution and Characters of Bacilli.—Tubercle bacilli are found in small numbers throughout the tissue. They are all intracellular and occur within intact epithelial cells, desquamated epithelial cells, cell fusions, and giant cells.

They are generally from 2 to 4μ long, straight, and not beaded.

Liver.

No tubercle bacilli have been found in the liver. The absence of bacilli in this organ supports the view that those found in the lungs are due to inhalation.

SUMMARY.

There is a scanty invasion of tubercle bacilli in the lungs.

C.—SUMMARY.

The main feature brought out by these examinations is that the rat exhibits a remarkable degree of tolerance towards the tubercle bacillus, both bovine and human. Bacilli may be swarming all over the body, although the amount of tissue damage is extremely slight. Their habit of multiplying within the tissue cells is very noticeable. But in order to propagate the bacillus within the rat it seems necessary, in most cases, to inoculate very large doses. Moreover, the bacilli do not induce a morbid process which can be histologically described as tuberculosis. The bacillus does not readily kill the rat because the rat is, to a large extent, indifferent to it, not because it evokes the conservative tissue reaction characteristic of the "retrogressive tubercle." The "resistance" of the rat is obviously high, but it is different in kind from the resistance offered by animals such as the bovine and rabbit. All the viruses investigated, including viruses of low virulence for bovines and rabbits, may, under favourable circumstances, multiply freely in the rat. The appearance of tubercle bacilli in the rat is often suggestive of leprosy bacilli.

CONCLUDING REMARKS.

The tissues of all the animals which I have examined demonstrate the essential unity of the morbid process set up, in animals susceptible to the disease, by all varieties of human and bovine bacilli which have been investigated. Different animals differ in their susceptibility to viruses of different degrees of virulence, and these differences naturally find their histological expression. But of a difference in kind between these viruses there is absolutely no indication, and it appears to me that my histological investigations have been sufficiently comprehensive to justify my opinion that differences in kind between the morbid processes set up in mammals by human tubercle bacilli on the one hand and by bovine tubercle bacilli on the other do not exist.

ARTHUR EASTWOOD.

PART III.

THE COMPARATIVE STUDY OF TUBERCLE BACILLI FROM BOVINE AND HUMAN SOURCES.

THE COMPARATIVE STUDY OF TUBERCLE BACILLI FROM BOVINE AND HUMAN SOURCES.

By A. Eastwood.

INTRODUCTION.

This Report deals with the cultural characters of tubercle bacilli from bovine and human sources, and with the microscopic appearances which the bacilli exhibit in films from cultures and in smears and sections from animal tissues.

My culture work commenced in June, 1904, on the completion of the new bacteriological wing of my laboratory. This Report embodies work recorded up to August, 1906.

As the work necessitated the daily handling of large numbers of cultures from both sources, special precautions were taken to avoid the risk of accidental confusion. Separate rooms were set apart for the labelling, unsealing, and subculturing of the two sorts of viruses; and the tubes and flasks received distinctive labels and were incubated in separate rooms.

The starting points for my investigation consisted of material sent to me by my colleagues from the two experimental farms. With the exception of a few instances, when they sent me pieces of tissue from infected animals, they provided me with pure cultures which they had already isolated. The cultures from the bovine viruses were isolated by Drs. A. S. and F. Griffith; the cultures from the human viruses were isolated by Drs. L. Cobbett and H. J. Hutchens. I feel under a deep personal obligation to my four colleagues for their unfailing kindness in supplying me with all the cultures I wanted. I also feel very greatly indebted to them for their generosity in giving me the benefit of their valuable experience in the methods of cultivating tubercle bacilli. When I commenced my work, Dr. A. S. Griffith had completely overcome the initial difficulty of isolating and cultivating the bovine bacillus on artificial media; and my colleagues at the other experimental farm were also able to provide me with a culture of every variety of human bacillus they had encountered.

Method of Comparison.

Material Received.

My colleagues have provided me with strains representative of every important virus they have dealt with.* In the case of many of the more interesting viruses they have sent me several strains; and whenever differences were suspected or established between different strains belonging to the same virus, they have provided me with examples of each of these possible varieties.

Some of the cultures had been kept up on artificial media for a considerable time before I commenced my work, but in none of these cases was there any evidence that the cultural characters had been artificially modified before I investigated them. Whenever possible I received either a primary culture or a sub-culture of an early generation.

The bacilli sent to me had been grown since their isolation from the animal body on pure serum, except in certain cases where Drs. A. S. and F. Griffith had raised their primary culture on an egg medium.

Cultures for Comparative Purposes.

In order to secure validity of comparison I have adopted the following system :—

1. As pure serum has been found less liable than any other artificial medium to alter the natural characters of the tubercle bacillus, all my strains have been kept up on this medium.
2. When other media are inoculated, the inoculations are always made from cultures on pure serum.
3. Every batch of every medium prepared receives a distinctive label and is apportioned equally between the viruses from the bovine and those from the human experimental farm.
4. The qualities of all culture media, even when prepared in the same way and from the same kind of material, are liable to variation; this is particularly the case with sera and with potatoes. I have made it a rule, therefore, not to pronounce a growth poor on any one kind of medium unless it has been found that several other strains, when grown on the same batch of the same medium, have yielded good growths.
5. Special importance is attached to inoculating from young and vigorous cultures. The character of the sub-culture depends to an important extent on the age and vigour of the culture from which it is obtained. The material sent to me is therefore not transferred to media for comparative purposes until I have found it growing well or satisfactorily on my own serum tubes.
6. Identity of technique is observed in the inoculation, sealing and incubation of all cultures.

Technique.

Preparation of Media.

Serum.—I am indebted to my colleagues for blood from normal bovines, pigs, and dogs, and for some of my horse's blood. The blood has been allowed to clot and the serum has then been syphoned off. The greater part of my horse serum has been obtained from the Wellcome Physiological Research Laboratories. This serum was first obtained as plasma, by means of potassium oxalate, from normal horses. The plasma was subsequently clotted after the addition of the necessary amount of calcium chloride. I find there is no very important difference for cultural purposes between the horse serum obtained in this way and the horse serum obtained by syphoning off, after the blood has clotted in the ordinary way. After being received into culture tubes, the serum has in many cases been subjected to fractional sterilisation for three days in succession at 56°, and then coagulated on the fourth day at from 72° to 75°, in an atmosphere saturated with moisture. Sometimes I dispense with the preliminary heating at 56°, and coagulate the serum at once. Exposure to a temperature of from 75° to 80° on two successive days has not been found to impair the nutritive value of the serum. This last method was suggested to me by Dr. A. S. Griffith. When the coagulated serum has cooled, the plugs are sealed with paraffin and the tubes are then stored until required for use.

Glycerinated Serum.—The serum is diluted with 5 per cent. of pure glycerin, and the medium is then prepared in the same way as pure serum.

Broth, Glycerin-Agar and Potato.—These media are prepared in the ordinary way, with the addition, in each case, of 5 per cent. of glycerin. The acidity of the broth to phenolphthaleine is from + 12 to + 16 per litre.

* We have adopted a convenient distinction between the words "strain" and "virus." All bacilli ultimately derived from the same human or bovine subject, viz., material from naturally infected bovines sent from the slaughter-house, or material from infected human subjects sent from the hospitals, are said to belong to the same "virus." Cultures have been raised sometimes from this original material and, in other cases, from experimental animals infected with this material either directly or after previous animal passage. Each of these cultures is regarded as the starting point of a separate "strain."

Inoculation.

The naked eye appearance of a subculture depends to an important extent on the amount of material transferred, its degree of moisture or dryness, and the method of its distribution. All my inoculations are done with a small platinum loop, which is only once charged. The loop is first passed into the condensation water at the bottom of the tube, and then gently drawn upwards, until a moderate amount of culture adheres to it. This material is then spread very thoroughly, and as far as possible evenly, over the entire surface of the serum, agar, or potato tube to be inoculated; to facilitate its distribution the loop is frequently dipped into the condensation water or glycerin solution during the process.

To cultivate the tubercle bacillus on broth I have devised a method which, so far as I know, is new. A layer of serum is inspissated on one of the narrow sides of an ordinary medicine flask. During the process of coagulation, the medicine flask, instead of resting upright on its narrow side, is tilted at a slight angle from the perpendicular, in order to give the serum a suitable slope. After cooling, a convenient amount of broth is poured into the flask, which is then laid on the broad side corresponding to the broad end of the wedge of the serum. The serum slope above the level of the broth is inoculated in the usual way. The bacilli grow down the serum slope, and when they reach the broth, have the opportunity of growing over its surface. This seems to me a more reliable method than any other I know for objectively estimating the relative readiness or reluctance of different strains of tubercle bacilli to grow on a liquid medium.

Incubation.

Before being placed in the incubators all culture tubes and broth flasks are sealed with paraffin. The culture tubes are kept in an inclined position, at an angle only slightly above the horizontal. The temperature of the incubators is $37\frac{1}{2}°$.

Observation.

Every tube or flask inoculated has been examined at least once a week, and notes have been recorded of its progress. These observations are summarised and embodied in the detailed descriptions contained in the present report.

Staining of Films.

Films are stained in carbol-fuchsin for 20 minutes, with steam gently rising. They are then treated with 25 per cent. H Cl and counterstained with methylene-blue.

METHOD OF RECORDING CULTURAL CHARACTERS.

The main object of this part of my work is to form an independent opinion upon the classification of tubercle bacilli isolated from the bovine and from man. I begin by taking each strain as it comes, and work out its cultural characters as though it might conceivably be a new organism, without reference to any preconceived hypothesis. I have noted the rapidity and amount of growth of the bacilli on various media, together with their naked-eye and microscopic appearances. Subsequently, I base on these details a classification, according to cultural characters, of the bacilli dealt with.

In the case of cultures raised on pure serum it soon became apparent that the naked-eye appearances of the growths did not, as a rule, present any differences which could be regarded as of much significance. In describing the naked-eye appearances of these cultures I have therefore generally been content to note the main fact which was of practical interest, the chief importance of serum cultures being to perpetuate the bacilli and to provide vigorous growths suitable for transference to other test media. A "good" growth means a growth in which it is obvious, from its amount and its ready formation, that the bacilli are in an actively growing condition, and therefore suitable for testing on other media which may or may not affect their vitality. A "satisfactory" growth means a growth in which the yield, though hardly large enough to be called "good," is yet sufficient to show that the bacilli are in a normal, readily growing condition, and that therefore they may be safely used for sub-culture on to test media. A "poor" growth is one in which I am not satisfied that this condition of normal cultural vigour has been obtained.

THE DETAILED RECORDS OF CULTURAL CHARACTERS.

VIRUS B I.

(Bovine Lung.)

(1.)

Strain.—G.P. 14 from ORIGINAL MATERIAL.

Material Received.—Fourteenth generation, 17 days old, of culture isolated from G.P. 14, which had been inoculated with original material.

On Horse Serum.—Cultures grew well; a fairly dense grey layer covered the surface uniformly at the end of a week. During the second and third weeks the growth increased in density and formed numerous small, raised grey colonies. In a 14 days' film the bacilli were uniformly stained and, with few exceptions, straight; they varied in length from 1·5 to ·75μ, with occasional still shorter forms; the average length was rather less than 1μ. In a 28 days' film of the same culture the only difference noted was that short forms (·75μ and less) were more numerous.

On Bovine Serum.—Growth, on the whole, as on horse serum. Some cultures, grown on particularly good samples of bovine serum, yielded denser growths than the horse serum cultures. In a film from one of these, 14 days old, some of the bacilli were rather longer than those grown on horse serum; these longer forms measured from 2 to 2·5μ; some of the bacilli exhibited darkly stained globular swellings.

On Dog Serum.—The first culture made on this medium produced a good growth, which was dull grey and uniform until the end of the third week and then began to develop discrete white colonies. A second and a third generation were grown on the same medium. They were both more abundant than the first, exhibiting white heaped-up colonies and patches which began to make their appearance at the end of the first week. The morphology of the bacilli, in 14 days' films, was identical in all three generations. They were short, straight, uniformly stained, and averaged about ·75μ in length.

On Glycerinated Bovine Serum.—Little, if any, growth took place during the first fortnight; at the end of the third week there was a marked increase, and numerous grey colonies had appeared. Some slow increase continued until the end of the sixth week. The ultimate yield was a moist, scanty growth consisting of moderately large, discrete, greyish yellow colonies. The surface between the colonies was free from growth. In a 21 days' film the bacilli measured from ·75 to 1·25μ; a few were curved; a few exhibited globular swellings.

On Broth.—Broth cultures were only obtained with difficulty. In the first two flasks inoculated a few minute delicate islands made their appearance between the third and fourth weeks, but failed to increase subsequently. In a third flask minute islands commenced to form at the beginning of the third week. Only one-third of the surface was covered at the end of five weeks, and it was not until the end of the seventh week that the surface was completely covered. The pellicle exhibited some opaque spots and patches, but was, on the whole, very delicate and semi-translucent. In a 44 days' film the bacilli measured from ·75 to 1·5μ. They were all straight and uniformly stained.

On Glycerin-Agar.—Growth, consisting of no more than a fine, grey, semi-translucent haze, appeared over the surface about the commencement of the second week. This growth exhibited hardly any increase until about the seventh week. About that time several minute lumps of material originally inoculated began to expand into grey colonies, slightly raised in the centre. These isolated colonies increased slowly for two or three weeks, and then their growth ceased. In a 73 days' film the majority of the bacilli were straight; some of them contained darkly stained, globular swellings, and about one-fourth were regularly beaded. The length of the bacilli varied from ·75 to 2μ and averaged about 1μ.

On Potato.—Some small, isolated, grey colonies appeared during the second week. These showed no visible increase until about the sixth week. During the seventh and eighth weeks a slight increase took place, and then growth ceased. In a 26 days' film the bacilli were straight, or only slightly curved; some of them stained regularly or faintly. They varied in length from 1·5 to 2μ.

SUMMARY. *

Growth good on serum, poor on glycerinated serum, very poor on broth, glycerin-agar, and potato.

(2.)

Strain.—Cow 44.

Material Received.—Third generation, 20 days old, of culture isolated from Cow 44.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were all uniformly stained and almost all straight. They varied in length from 1·5 to rather less than ·75μ, the average length being slightly less than 1μ. In a 28 days' film from the same culture the bacilli showed no alterations in morphological characters.

On Bovine Serum.—Growth as on horse serum. Bacilli slightly longer than on horse serum.

On Dog Serum.—Grew well; uniformly grey until the end of the third week; then white colonies and patches appeared. In a 14 days' film the bacilli were uniformly stained, nearly all straight, and on the average slightly under 1μ in length.

On Glycerinated Bovine Serum.—During both the first and the second week there was a definite but very slight increase. Considerable advance was made during the third week, and some further progress was made during the fourth week. The growth then ceased. The ultimate yield consisted of thin, irregular, grey, slightly raised patches. In a 21 days' film the bacilli measured from ·75 to 1·25μ; with the exception of a few which were curved and a few which exhibited globular swellings, they were all straight and uniformly stained.

On Broth.—After a delay of about a fortnight growth

* This and the following summaries are intended to give the reader a general notion of the characters of any particular strain, without requiring him to peruse a mass of detail. It is necessary for me to record these details, just as it is requisite for the morbid anatomist to describe, as an adequate and independent record, every *post-mortem* examination he makes. And, as in important anatomical studies, I think naked eye appearances should be systematically supplemented by a statement of microscopic characters. Moreover, since some of my general conclusions concerning cultural characters do not completely accord with conclusions formulated by other observers, it is desirable that I should state fully the observations on which my conclusions are based.

commenced and then progressed with moderate rapidity, the surface being covered in about five weeks. The pellicle was of unequal density; some patches were grey and moderately dense, but not raised into warty elevations; these patches thinned out at their periphery and merged into the rest of the pellicle, which was thin, homogeneous, and slightly opaque. In a 54 days' film the bacilli measured from 1 to $2 \cdot 5\mu$ in length, the majority being rather under 2μ; with the exception of a very occasional curved, beaded bacillus, the bacilli were straight and uniformly stained.

On Glycerin-Agar.—A scanty growth, consisting of no more than a fine grey haze, appeared during the first fortnight. During the seventh and eighth weeks the growth became slightly but uniformly denser, and a few large grey colonies developed near the bottom of the tube. In a 73 days' film the bacilli were straight and often irregularly stained. They varied in length from 1 to 2μ.

On Potato.—Some small grey colonies formed during the first fortnight. No further visible increase took place until the sixth week. The colonies then became larger, but did not increase in number, nor did the growth become confluent. In a 26 days' film the bacilli were straight and often irregularly stained; some showed regular beading. The bacilli varied in length from 1 to 3μ.

SUMMARY.

Growth good on serum; on glycerinated serum confined to irregular patches; on broth readily obtained but not dense; on glycerin-agar and potato very poor. On the whole, slightly, but distinctly, better than G.P. 14.

(3.)

Strain.—CALF 122.

Material Received.—Third generation, 41 days old, of culture isolated from Calf 122.

On Horse Serum.—Good growth. In a 15 days' film the bacilli were straight and uniformly stained; they measured from $\cdot 5$ to $1 \cdot 25\mu$. The average length was about $\cdot 75\mu$.

On Bovine Serum.—Growth good. Morphology, in a 17 days' film, as on horse serum.

On Broth.—Growth commenced on the broth about the end of the fourth week. The surface was covered at the end of the seventh week. The pellicle was delicate, semi-translucent, and speckled. In a 50 days' film the bacilli were straight, uniformly stained, and measured from 1 to $1 \cdot 5\mu$, occasionally 2μ.

On Glycerin-Agar.—Growth very poor. At the end of seven weeks there was nothing but a very delicate, greyish, semi-translucent film. During the eighth week a few pin-point grey specks appeared. In a 51 days' film the bacilli were straight, uniformly stained, and measured from $\cdot 75$ to $1 \cdot 5\mu$.

On Potato.—There were some signs of growth during the third week. During the fourth week definite, greyish streaks were observed. The growth then remained almost stationary. In a 55 days' film the bacilli measured from $\cdot 75$ to 3μ. The longer forms were generally curved. Except a relatively small number which were regularly beaded, the bacilli were uniformly stained.

SUMMARY.

Growth good on serum, poor on other media. On the whole, little, if any, better than G.P. 14.

(4.)

Strain.—BABOON 8.

Material Received.—Ninth generation, 19 days old, of culture isolated from Baboon 8.

On Bovine Serum.—Growth satisfactory. In an 18 days' film the bacilli measured from 1 to $1 \cdot 5\mu$, and occasionally as much as $2 \cdot 5\mu$. The average was rather over 1μ. They were straight and uniformly stained.

On Broth.—Growth commenced at the beginning of the second week, and the surface was covered at the beginning of the fifth week. The pellicle was very delicate in the earlier stages of growth, but afterwards exhibited irregular, flaky patches of denser material. In a 14 days' film the bacilli measured from 1 to $2 \cdot 5\mu$ and were straight and uniformly stained.

On Glycerin-Agar.—The increase was slow, but steadily maintained. At the end of three weeks there was a fine ground-glass appearance, with some minute raised grey points. This growth slowly increased in density and roughness, and at the eighth week there was a typical frosted glass appearance; the raised points were more numerous and larger, and the growth had spread partially over the glass. In a 29 days' film the bacilli measured from $\cdot 75$ to 3μ; they were uniformly stained; a few were curved.

On Potato.—A very slight amount of increase was observed during the first fortnight and then ceased. In a 36 days' film the majority of the bacilli resembled those grown on serum, but there were some longer forms.

SUMMARY.

Compared with other strains of this virus, there is a marked increase of luxuriance on glycerin-agar.

(5.)

Strain.—BABOON 64.

Material Received.—Primary culture, 62 days old, isolated from the lungs of Baboon 64. This animal had been fed with culture of Baboon 8.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from $\cdot 5$ to $1 \cdot 5\mu$, and were straight and uniformly stained.

On Broth.—Many grey islands appeared during the second week and the surface was nearly covered at the end of a month. The pellicle then became moist and sank. In a 27 days' film the bacilli varied in length from 1 to 3μ. Most of them were straight; irregularly stained forms were rare.

On Glycerin-Agar.—No more than a grey haze with a few denser points was formed in two months. In a 42 days' film the bacilli varied in length from $\cdot 75$ to 3μ and were nearly all uniformly stained. A few curved forms were noted.

On Potato.—A scanty, yellowish growth was slowly formed. In a 56 days' film the bacilli varied in length from $\cdot 75$ to 5μ, or occasionally longer. They were nearly all curved and were either beaded or showed irregular thickenings.

SUMMARY.

Growth much poorer than the strain through Baboon 8, particularly on glycerin-agar.

(6.)

Strain.—BABOON 66.

Material Received.—Second generation, 10 days old, of culture isolated from the spleen of Baboon 66.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from ·75 to 1·5μ and were straight and uniformly stained.

On Broth.—Growth slow, irregular, and of unequal density. The surface was about half covered at the end of six weeks and then became moist and sank. In a 40 days' film the bacilli resembled those grown on serum, but some longer forms were noted.

On Glycerin-Agar.—No more than a grey haze with a few denser points was obtained. In a 42 days' film the bacilli resembled those grown on serum.

On Potato.—A very scanty, white growth was found at the end of two months. In a 56 days' film the bacilli varied in length from ·75 to 4μ. The shorter forms were straight and irregularly stained. The longer forms were generally slightly curved and stained uniformly.

SUMMARY.

Growth poor on broth and very poor on glycerin-agar.

(7.)

Strain.—MONKEY 80.

Material Received.—Tenth generation, 9 days old, of culture isolated from Monkey 80.

On Bovine Serum.—Growth satisfactory. In a 21 days' film the bacilli measured from ·75 to 1·5 and occasionally 2μ; they were uniformly stained and nearly all straight.

On Broth.—A few small islands appeared at the end of the second week. During the third week their increase was slow. During the fourth week they spread rapidly and covered the surface. The pellicle was very thin and delicate to begin with, but at the end of the month had thickened slightly and become semi-opaque; it then became moist and a good deal of it sank. In a 31 days' film the bacilli varied in length from 1 to 3μ. Most of them were straight and uniformly stained, but some exhibited globular thickenings.

On Glycerin-Agar.—Very little increase took place during the first three or four weeks; then some improvement occurred, and by the eighth week the surface was covered with a rather thin, grey layer which had spread on to the glass and was interspersed with numerous small, grey, nodular points. In a 47 days' film the bacilli varied in length from 1 or 1·5 to 4μ; many were curved, and many were regularly beaded.

On Potato.—Some increase was noted during the second and third weeks, but there was no subsequent progress, the total yield at the end of eight weeks being a very scanty, non-pigmented layer. In a 50 days' film the bacilli measured from 1 to 3μ; most of them were straight; beading was rather frequent.

SUMMARY.

Growth not so good as Baboon 8. but better than (1), (2), or (3).

(8.)

Strain.—MONKEY 82.

Material Received.—Eighth generation, 9 days old, of culture isolated from Monkey 82.

On Bovine Serum.—Growth good. In a 21 days' film the bacilli measured from ·5 or ·75 to 1·25 or 1·5μ; they were straight and uniformly stained.

On Broth.—Growth commenced at the end of the third week and then advanced slowly during the three subsequent weeks. The pellicle was thin, slightly opaque, and moist. It began to sink before it had succeeded in covering the surface. In a 34 days' film the bacilli measured from 1 to 2·5μ, and were nearly all uniformly stained; some of the longer forms were curved.

On Glycerin-Agar.—At the end of three weeks the growth consisted of a fine, nearly translucent layer interspersed with some small grey points. During the five or six following weeks these points gradually increased in elevation and extension, but no change was observed in the rest of the surface. In a 27 days' film the bacilli varied in length from 1 to 3μ; nearly half were curved; many were regularly beaded; forms with globular swellings were less numerous.

On Potato.—A scanty, non-pigmented growth developed during the first three weeks and then ceased. In a 50 days' film the majority of the bacilli resembled those grown on serum, but there were some beaded and some irregularly stained forms.

SUMMARY.

Growth rather inferior to Monkey 80.

(9.)

Strain.—PIG 8.

Material Received.—Eleventh generation, 9 days old, of culture isolated from Pig 8.

On Bovine Serum.—Growth very good. In a 21 days' film the bacilli measured from 1 to 2·5μ, being longer than is usual for a serum culture. Curved forms were common, globular swellings were fairly numerous, and beaded forms were occasionally found.

On Broth.—Growth commenced at the end of the third week, and the surface was almost covered at the end of the sixth week. The pellicle was unequal, being thin in parts, but mottled with denser streaks and patches. In a 35 days' film the bacilli measured from ·75 or 1 to 2·5μ. They were straight, and, with the exception of an occasional globular swelling, uniformly stained.

On Glycerin-Agar.—No more than a fine grey haze was obtained. In a 47 days' film the bacilli measured from 1 to 3·5μ; many were curved, and many beaded; there were a few globular swellings.

On Potato.—A slight but definite increase was observed during the second and third weeks. There was no subsequent change. In a fifty days' film the bacilli resembled those grown on serum.

SUMMARY.

Growth about equal to that of Cow 44.

(10.)

Strain.—Pig 96.

Material Received.—Sixth generation, 6 days old, of culture isolated from Pig 96.

On Horse Serum.—Growth good. In a 16 days' film the bacilli varied in length from ·5 to 1·5μ, averaging under 1μ. They were straight and uniformly stained.

On Broth.—Growth was obtained slowly and with difficulty. Minute islands were observed floating at the end of the fourth week, and at the end of the fifth week about two-thirds of the surface were covered with very thin, irregularly distributed, and almost transparent material. No further extension or thickening took place. In a 39 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—No more than a fine grey haze was obtained at the end of two months. In a 42 days' film the bacilli, for the most part, resembled those grown on serum, but a few longer, irregularly stained, or regularly beaded forms were noted.

On Potato.—No growth was visible at the end of the first month. At the end of the second month there was a scanty increase. In a 56 days' film the bacilli varied in length from 1·5 to 3·5, and occasionally 4μ. The longer forms were curved. The staining was generally uniform.

SUMMARY.

Growth very poor; no better than the strain through G.P. 14.

(11.)

Strain.—Pig 98.

Material Received.—Second generation, 40 days old, of culture isolated from Pig 98.

On Bovine Serum.—Growth rapid and abundant. In a 13 days' film the bacilli were found to be unusually long, for a serum culture. They measured from 1·5 to 3·5μ, and a few forms were found as long as 4 or 4·5μ. More than half the bacilli were curved. Many were irregularly stained; deeply stained thickenings were very common, and imperfect beading was sometimes seen.

On Broth.—Growth commenced at the end of the third week, and the surface was covered at the end of the sixth. The pellicle was unequal in consistency; some of it was very delicate and speckled with small grey points whilst other parts were much denser and warty. In a 39 days' film the bacilli measured from 1 to 2·5μ; they were uniformly stained; a few of the longer were curved.

On Glycerin-Agar.—At the end of five weeks the growth consisted of a thin, nearly transparent layer interspersed with some isolated, raised, grey nodules. Some extension of growth took place from these grey nodules until the end of the ninth week. In a 27 days film the bacilli varied in length from 1 to 3·5μ; about one-third were curved and about the same number were beaded.

On Potato.—A slight, non-pigmented layer developed during the first three weeks, and then growth stopped. In a 57 days' film the bacilli resembled those grown on serum, with the exception of a few rather longer and beaded forms.

SUMMARY.

Growth slightly better than Pig 8 and about equal to Monkey 80.

VIRUS—B II.

(Bovine Lung.)

(1.)

Strain.—Heifer 100.

Material Received.—Sixth generation, 15 days old, of culture from Heifer 100.

On Horse Serum.—On the first culture made (seventh generation) there was evidence of growth on the fifth day. On the 14th day there was a fairly dense layer covering the entire surface, and studded with small grey elevations; the growth was much increased on the 21st day; in 28 days there was a further increase, and the elevations were larger, tended to coalesce, and exhibited a yellowish tinge. Later cultures produced a good growth as early as the eighth day, and the ultimate yield was rather larger than in the first culture. In a 14 days' film the bacilli were uniformly stained and, with very few exceptions, straight. They varied in length from about 1·5μ to rather less than ·75μ, the average being under 1μ. In a 28 days' film of the same culture a few beaded forms were noted, but otherwise the morphological characters were not altered.

On Bovine Serum.—The first culture, inoculated on what turned out to be a poor medium for all viruses, grew scantily; later cultures all grew well. Microscopically, the only differences noted from the horse serum films were that forms as long as 2 to 2·5μ were somewhat frequent, and some of the bacilli exhibited a deeply stained globular body.

On Broth.—Small islands of delicate, almost transparent film were floating at the end of three weeks; the surface was completely covered with a film of the same type in from five to six weeks; after that period no further change or increase took place. When the serum was inoculated from a particularly vigorous culture, growth took place rather less slowly, but, with the exception of a few small, white, slightly raised flakes, was of the same character. In a 48 days' film the bacilli were short and straight; a few only were slightly curved; none were beaded. The length of the majority was from ·75 to 1·5μ; very few were as long as 2 or 2·5μ. The bacilli were equally stained, and very uniform in their character.

On Glycerin-Agar.—A fine grey haze developed during the first fortnight. No further change took place until near the end of the second month, when a few raised, grey colonies appeared. In a 63 days' film the majority of the bacilli were straight; a large number of them contained deeply stained globular swellings. The length of the bacilli varied from 1 to 3μ. A film taken from a very thin portion of the growth proved identical in microscopic characters with another which was taken from a raised colony.

On Potato.—Growth very scanty. In a 26 days' film the majority of the bacilli were straight, or only slightly curved, uniformly stained, and about 1·5μ in length. There were some irregularly or faintly stained forms and a few, from 3 to 4μ in length, which were curved and beaded.

SUMMARY.

Growth very poor, but somewhat better than two of the representatives of B.I, *viz.*, G.P. 14 and Calf 122.

(2.)

Strain.—CALF 134.

Material Received.—Fourth generation, 14 days old, of culture isolated from Calf 134.

On Horse Serum.—Good growth. In a 15 days' film the bacilli were straight, uniformly stained, and averaged a little over $\cdot 75\mu$ in length.

On Bovine Serum.—Grew well. In a 17 days' film the bacilli measured from $\cdot 5$ to $1\cdot 25\mu$ and were, on the whole, rather shorter than those grown on horse serum.

On Broth.—At the commencement of the third week there was a continuous pellicle streaming off the serum for a distance of about a quarter of an inch on to the surface of the broth. The surface was nearly covered in six weeks. The pellicle was thin, but opaque and homogeneous. In a 25 days' film the bacilli were all straight or only very slightly curved and were all uniformly stained. Their length was from 1 to 2μ.

On Glycerin-Agar.—At the end of a fortnight minute, moist, semi-transparent colonies were observed. At the end of three weeks some of these colonies had become grey but were only pin-point in size. During the five subsequent weeks a very slight increase took place. In a 51 days' film the bacilli measured from 1 to 3μ. The great majority were straight and uniformly stained. A relatively small number were curved. Some were faintly but uniformly stained, and a few were regularly beaded.

On Potato.—A definite growth, exhibiting several yellowish colonies, was first observed in the middle of the third week. The growth increased during the fourth week and became slightly raised. It never became abundant. In a 55 days' film the bacilli were straight, uniformly stained, and measured from $\cdot 75$ to $1\cdot 5\mu$.

SUMMARY.

Growth appreciably better than Heifer 100.

(3.)

Strain.—G.P. 1735 from SERIES OF G.P.S.

Material Received.—Fourth generation, 17 days old, of culture isolated from G.P. 1735. The bacilli in this animal had been obtained, by an unbroken series of passages through guinea-pigs, from the original material inoculated $3\frac{1}{3}$ years previously.

On Bovine Serum.—Growth good. In a 14 days' film most of the bacilli varied in length from about $\cdot 75$ to $1\cdot 5\mu$ and were straight and uniformly stained. A few exceptional forms were from 2 to 3μ in length, and were in many cases curved and irregularly stained.

On Broth.—Growth commenced at the end of the second week, but was not well maintained, the surface not being completely covered at the end of six weeks. At this period portions of the pellicle began to sink, and there was no further surface extension. The pellicle was thin, opaque, with some speckled islands, and rather moist. In a 22 days' film the bacilli varied in length from $1\cdot 5$ to 4 or $4\cdot 5\mu$. About one-third of them were curved. About half were irregularly stained; some showed globular swellings, and some were regularly beaded.

On Glycerin-Agar.—At the end of a fortnight the surface was covered with a greyish layer, amongst which were numerous small raised colonies. The layer gradually increased until the end of the fifth week; at that period it was still thin on the whole, but showed many slightly raised grey colonies and patches. No further change took place. In a 42 days' film the bacilli resembled, on the whole, those grown on serum, but their average length was rather greater.

On Potato.—There was a seanty white growth in three weeks; this became somewhat denser during the second month. In a 60 days' film the bacilli varied in length from 1 to 3μ. The great majority were straight and uniformly stained. A few globular swellings were noted.

SUMMARY.

Growth poor on broth, but on other test media better than Heifer 100 and about equal to Calf 134.

VIRUS—B III.

(Bovine Thoracic Glands.)

(1.)

Strain—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 17 days old, of culture isolated from original material.

On Horse Serum.—Grew readily and abundantly. In a 14 days' film the bacilli were straight and uniformly stained. They varied in length from $1\cdot 5$ to less than $\cdot 5\mu$, the average length being about $\cdot 75\mu$. In a 28 days' film of the same culture there was no appreciable difference in morphological characters.

On Bovine Serum.—The growth, on good samples of this serum, was rapid and abundant. In a 14 days' film the bacilli were slightly longer than those grown on horse serum, forms from 2 to $2\cdot 5\mu$ being met with; and some of the organisms contained darkly stained globular swellings.

On Dog Serum.—Grew abundantly, being uniform and dull grey until the third week, when white colonies began to appear. In a 15 days' film the bacilli were straight and uniformly stained, generally between $\cdot 75$ and 1μ in length, occasionally as long as $1\cdot 5\mu$.

On Glycerinated Bovine Serum.—Growth commenced very slowly, yielding only a slight semi-translucent layer at the end of a fortnight. During the third week there was a well-marked increase and the growth became grey. There was some further increase during the fourth and fifth weeks. The ultimate yield was moist and rather seanty; there were several fairly large whitish yellow colonies and also some irregular patches of thinner, continuous growth. In a 21 days' film the bacilli measured from $\cdot 75$ to $1\cdot 25\mu$; a few were curved and a few exhibited globular swellings.

On Broth.—Pellicle extremely delicate and almost completely transparent. It began to form in the third week and had covered the surface at the end of the sixth week. Though kept for a much longer period, no thickening took place. In a 61 days' film the bacilli were straight, with the exception of a few which were slightly curved; they were all uniformly stained. Their length varied from $\cdot 75$ to 2μ.

On Glycerin-Agar.—A fine grey haze only, with the appearance, at the end of seven weeks, of two or three grey colonies. In a 73 days' film the bacilli were almost all straight and uniformly stained; their length varied from $\cdot 5$ to 2μ, the average being under $1\cdot 5\mu$.

On Potato.—Growth very seanty and slow. A few minute white colonies. In a 26 days' film the majority of the bacilli were straight, uniformly stained, and about $1\cdot 5\mu$ in length. Some longer and irregularly stained forms were also noted.

SUMMARY.

Growth good on serum, poor on glycerinated serum, and very poor on other media.

(2.)

Strain.—Mouse 10, from Original Material.

Material Received.—Seventh generation, 21 days old, of culture isolated from Mouse 10.

On Horse Serum.—Good growth. In a 15 days' film most of the bacilli measured from ·75 to 1μ; a few forms were from 1·5 to 2μ; the bacilli were straight and uniformly stained.

On Bovine Serum.—Good growth. In a 17 days' film the bacilli were slightly shorter than those grown on horse serum.

On Broth.—A small gauze-like island speckled with white points appeared during the fifth week in one flask and during the third week in another. The total yield, at the end of two months, was poor, consisting of several small, irregular, semi-transparent, speckled islands. In a 20 days' film the bacilli measured from ·5 to 1·5, or occasionally 2μ, and were nearly all straight and uniformly stained.

On Glycerin-Agar.—Growth extremely scanty. A fine grey haze on the surface and a few pin-point grey colonies were all that was to be seen at the end of two months. In a 51 days' film the bacilli were straight, uniformly stained, and measured from ·75 to 1·5μ.

On Potato.—In three cultures made, very little, if any, growth had occurred at the end of two months.

Summary.

Growth, except on serum, extremely poor.

(3.)

Strain.—Monkey 74.

Material Received.—Fourth generation, 14 days old, of culture isolated from Monkey 74.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were uniformly stained and, with the exception of a relatively small number, straight. Their length varied from 2·5 to ·5μ, the average being about 1μ. In a 28 days' film from the same culture the bacilli were slightly longer. In a 14 days' film from another culture the bacilli averaged rather over 1μ, and a few beaded forms were noted.

On Bovine Serum.—Growth satisfactory. In a 14 days' film the bacilli resembled those in the first mentioned 14 days' horse serum film. In a 28 days' film from the same bovine serum culture no change was noted.

On Dog Serum.—Grew well; uniformly grey at first; white colonies and patches made their appearance during the third week. The bacilli, in a 14 days' film, were straight, uniformly stained, and averaged in length rather less than 1μ.

On Glycerinated Bovine Serum.—Very poor for the first fortnight; well marked increase during the third week, when numerous small white colonies appeared. Further increase during the fourth and fifth weeks. The final yield was moderately good; it consisted of a uniform layer of the ground glass type, speckled with numerous small white colonies. In a 15 days' film the bacilli measured from ·75 to 2·5μ, rarely 3μ; a few were curved; an occasional bacillus was regularly beaded; the rest were straight and uniformly stained.

On Broth.—In a month's time the surface was covered with a thin greyish film, speckled with a moderate number of slightly raised, opaque, pin-head points, and rather larger, irregular patches. In a 28 days' film the bacilli were found to be highly uniform. They were all straight and not beaded, and ranged in length from 1 to 2μ, the average being about 1·5μ.

On Glycerin-Agar.—A fine grey haze. About the sixth week a few denser patches began to appear. In a 46 days' film the bacilli were nearly all uniformly stained. They measured from ·5 to 2·5 in length, with the exception of the larger forms, which were relatively few, they were all straight.

On Potato.—Growth very slow, scanty, and non-pigmented. In a 25 days' film the bacilli measured from ·75 to 2·5μ, and were nearly all straight. Some stained faintly, and a few were regularly beaded.

Summary.

Growth poor, but somewhat better than the two former strains of this virus.

VIRUS—B IV.

(Bovine Mesenteric Glands.)

(1.)

Strain.—Original Material.

Material Received.—Seventh generation, 20 days old, of culture isolated from original material.

On Horse Serum.—Grew readily and abundantly. In a 14 days' film the bacilli were all uniformly stained and almost all straight. Some unusually slender forms were noted. The bacilli varied in length from 1·5 to ·5μ, the average being between ·75 and 1μ.

On Bovine Serum.—Growth as on horse serum. In a 14 days' film the bacilli were slightly longer than on horse serum, and slender forms, though present, were less numerous.

On Dog Serum.—Grew well; uniform and grey for the first fortnight, white and elevated afterwards. In a 15 days' film the bacilli measured from ·75 to 1·5μ and occasionally 2μ; they averaged about 1μ. They were all uniformly stained. Only a few were curved.

On Glycerinated Bovine Serum.—Many small discrete colonies were formed at the end of a week. At the end of a fortnight groups of these colonies had coalesced and formed irregular grey patches. Some further increase took place during the third and fourth weeks, but the total yield was scanty and irregularly distributed. In a 15 days' film the bacilli measured from 1 to 3μ. The majority were uniformly stained and more than three-fourths were straight. A few were regularly beaded and some contained globular swellings.

On Broth.—In a month's time the entire surface was well covered with a delicate gauze-like film overlaid with numerous dense, white, somewhat raised spots and patches. In a 28 days' preparation the bacilli exhibited marked uniformity. All were deeply and evenly stained, straight or only very slightly curved, and measured from 1 to 2μ, the average being about 1·5μ.

On Glycerin Agar.—A fine grey haze formed during the first fortnight. During the fifth week a few minute grey colonies appeared; these slowly increased during the three following weeks. In a 46 days' film the bacilli were nearly all evenly stained and varied in length from 1 to 1·5μ; a few were as long as 3·5μ. About one-fifth of the bacilli were curved.

On Potato.—It was doubtful if there was any increase of the material inoculated.

Summary.

Growth on serum good, on glycerinated serum poor; on broth a pellicle of irregular density was readily obtained; growth on other media very poor.

(2.)

Strain.—RAT 42 from ORIGINAL MATERIAL.

Material Received.—Third generation, 8 days old, of culture isolated from G.P. 1597, which was inoculated from Rat 42.

On Bovine Serum.—Growth good. In a 12 days' film the bacilli varied in length from ·75 to 1·5μ. They were straight and uniformly stained; some rather slender forms were noted.

On Broth.—Growth commenced at the end of the third week, and the surface was nearly covered at the end of the sixth week. The pellicle was, with the exception of a few small, grey circular areas, transparent, extremely thin, and rather fragmentary. It failed to show any increase in thickness after being kept until nearly three months old. In a 47 days' film the majority of the bacilli resembled serum-grown bacilli but were, perhaps, slightly longer. Regularly beaded forms occasionally occurred, and there were a few curved and beaded bacilli as long as 3·5μ.

On Glycerin-Agar.—A very thin, only slightly opaque, grey layer was formed at the end of a fortnight. This very gradually became a little denser, and at the end of eight weeks formed a thin, uniform, definitely opaque, grey layer, exhibiting on the surface a "broken glass" appearance. In a 42 days' film the bacilli varied in length from 1 to 3μ. The majority were straight and uniformly stained.

On Potato.—A slight increase was observed at the end of a fortnight, and at the end of eight weeks this had become a rather thin, evenly distributed, non-pigmented layer. In a 51 days' film the bacilli varied in length from 1 to 3μ. Most of them were uniformly stained. A few were curved.

SUMMARY.

Growth on the whole, slightly better than the culture from the original material.

(3.)

Strain.—CAT 12 from COW 164.

Material Received.—Second generation, 10 days old, of culture isolated from Cat 12, which had been fed with the organs of a G.P. inoculated with the milk of Cow 164.

On Bovine Serum.—Growth abundant and rapid. In a 13 days' film the bacilli were longer and more varied than is usual in serum cultures. The length varied from 1 to 3·5 and occasionally 4μ. The forms about 1μ in length or a little over were straight, rather thick, and uniformly stained. The longer forms were generally very slender and frequently curved. Often part of the bacillus was stained very faintly and the rest deeply, the more deeply stained part being rather thicker than the rest of the bacillus but not globular. A few bacilli were regularly beaded

On Broth.—Growth commenced at the end of the third week and the surface was covered with a very thin, delicate film at the end of the sixth week. In a 39 days' film the bacilli varied in length from ·75 to 2μ and were straight and uniformly stained.

On Glycerin-Agar.—A thin, grey haze was formed, with slightly denser patches. In a 47 days' film the bacilli varied in length from 1 to 3 and occasionally 4μ. The majority were straight and uniformly stained, but a small proportion were curved, and some were regularly beaded.

On Potato.—A slight increase was obtained in three weeks, and then the growth stopped. In a 57 days' film the bacilli, with the exception of an occasional longer beaded form, varied in length from ·75 to 2μ, and were straight and uniformly stained.

SUMMARY.

Growth poor on broth and on other media not appreciably better than the culture from the original material.

(4.)

Strain.—DOG 18 from COW 164.

Material Received.—Fifth generation, 19 days old, of culture isolated from Dog 18, which had been fed with the organs of a G. P. inoculated with the milk of Cow 164.

On Bovine Serum.—Growth good. In an 18 days' film the bacilli varied in length from ·75 to 1·5μ and were straight and uniformly stained.

On Broth.—Growth commenced about the end of the second week. It was thin at first but afterwards became thick and opaque. Though increasing in thickness, the surface extension of the pellicle was very slow, the broth being only two-thirds covered at the end of six weeks. In a 14 days' film the bacilli varied in length from 1 to 3μ. Many of them were curved; there were many globular swellings, and there was some indistinct beading.

On Glycerin-Agar.—There was a steady and moderately rapid increase. At the end of three weeks the surface was covered with an opaque, fairly dense, and uniform layer. During the fourth week the surface was thrown into minute flakes, giving it a frosted-glass appearance, and some wrinkles were formed. At the end of eight weeks the growth was thick and very wrinkled. In a 29 days' film the bacilli varied in length from 1 to 4·5μ, the average being about 2·5μ. The great majority were curved. A moderate number were regularly beaded; the staining was otherwise uniform.

On Potato.—A scanty increase was observed during the first month, with the formation of pale yellow pigment. In a 36 days' film there were a few short, straight forms (1 to 1·5μ) whilst the rest measured from 2 to 5μ and were curved, slender, and more or less regularly beaded.

SUMMARY.

On both broth and glycerin-agar there was a remarkable increase of luxuriance, compared with previous strains of this virus. On potato the growth was pigmented.

(5.)

Strain.—DOG 92 from DOG 54.

Material Received.—Second generation, 19 days old, of culture isolated from the lung of Dog 92.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from ·5 to 1·5μ and were straight and uniformly stained.

On Broth.—The surface was covered in a fortnight with a thin, opaque, homogeneous pellicle. The pellicle remained floating for several weeks without either thickening or showing any tendency to sink. In a 27 days' film the bacilli resembled those grown on serum, but were slightly longer.

On Glycerin-Agar.—A grey layer was formed at the end of a week and steadily increased until the end of the fifth week. The ultimate yield was a thick, dull grey, dry, nearly uniform layer, with no prominent wrinkles or warts. In a 41 days' film the bacilli varied in length from 1 to

$3\cdot5\mu$, with occasional longer and shorter forms. They were uniformly stained and often curved.

On Potato.—Patches of grey growth appeared during the second week, and afterwards became cream coloured. The total growth was moderately abundant. In a 56 days' film the bacilli varied in length from 1 to 5μ and were for the most part curved and irregularly stained or regularly beaded.

SUMMARY.

A decided increase in luxuriance, particularly on glycerin-agar, compared with other strains of B IV.

(6.)

Strain.—DOG 116.

Material Received.—Primary culture, on egg, 14 days old, of culture isolated from the lung of Dog 116.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from $\cdot75$ to $1\cdot5\mu$ and were straight and uniformly stained.

On Broth.—The surface was nearly covered in about five weeks with a thin, rather opaque pellicle. There was no particular tendency for the pellicle to become moist. In a 12 days' film the bacilli varied in length from 1 to 3μ. A few were curved. The great majority were uniformly stained.

On Glycerin-Agar.—The growth was slow and scanty, thin on the whole, but exhibited some denser patches. In a 38 days' film the bacilli varied in length from 1 to 3μ. Curved forms were not very common. A moderate number of bacilli were beaded.

On Potato.—A scanty, white growth was slowly formed. In a 38 days' film the bacilli varied in length from $\cdot75$ to $3\cdot5\mu$. Many were curved, and irregularly stained orms were fairly common.

SUMMARY.

Growth poor on the whole; much poorer than either Dog 92 or Dog 18, but better than other strains of B IV.

(7.)

Strain.—COW 172.

Material Received.—Primary culture, 19 days old, isolated from Cow 172.

On Bovine Serum.—Growth poor in the first two subcultures, afterwards satisfactory. In a twelve days' film the bacilli varied in length from $\cdot75$ or 1 to 2 or $2\cdot5\mu$. They were uniformly stained. A moderate number of them were curved.

On Broth.—Growth commenced in the third week and the surface was covered at about the end of the fifth week. The pellicle consisted of a semi-transparent layer, beset with numerous, raised, warty patches. In a 41 days' film the bacilli resembled those grown on serum, but some slightly longer forms were noted.

On Glycerin-Agar.—Growth scanty; a grey haze interspersed with some denser patches. In a 43 days' film, the bacilli varied in length from 1 to 3μ. Most of them were straight. A few were beaded or stained irregularly.

On Potato.—A few pale colonies appeared in the third week and afterwards became rather larger. In a 51 days' film the bacilli varied in length from 1 to $3\cdot5\ \mu$. Curved forms were rather rare. Most of the bacilli were uniformly stained, but there were a few globular swellings.

SUMMARY.

Growth similar to the culture isolated from the original material, or perhaps slightly better.

(8.)

Strain.—BABOON 22.

Material Received.—Fifth generation, 9 days old, of culture isolated from Baboon 22.

On Bovine Serum. Growth abundant. In a 21 days' film the bacilli varied in length from 1 to 2 or $2\cdot5\mu$, being slightly longer than is usual for serum cultures. Most of them were straight. There were a considerable number with globular swellings.

On Broth.—Growth commenced in the third week, and the surface was covered at the end of the fifth week. The pellicle was very delicate at first; it increased slightly in opacity, and was speckled with small grey elevations. In a 31 days' film the bacilli varied in length from 1 or $1\cdot5$ to 3μ. The majority were straight. Beading and globular swellings were rather frequent.

On Glycerin-Agar.—A fine grey haze was all that was formed at the end of eight weeks. In a 27 days' film the bacilli varied in length from $1\cdot5$ to $3\cdot5\mu$. Most of them were curved and beaded.

On Potato.—A definite but scanty, non-pigmented layer was observed to form during the second and third weeks. No increase took place subsequently. In a 50 days' film the bacilli resembled those grown on serum, with the exception of a few rather longer and a few beaded forms.

SUMMARY.

Growth, except on serum, poor. No better than the strain through Cat 12.

(9.)

Strain.—BABOON 62.

Material Received.—Primary culture, 44 days old, isolated from the spleen of Baboon 62.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from $\cdot5$ to $1\cdot5\mu$. They were straight and uniformly stained.

On Broth.—Small, delicate islands were formed during the third week. Subsequent growth was slow, and the surface was not more than half covered at the end of 2 months. In a 42 days' film the majority of the bacilli were no longer than serum bacilli, but some measured

from 3 to $3 \cdot 5\mu$. Most of the bacilli were straight. Their staining was often irregular.

On Glycerin-Agar.—A grey haze, with a few denser points, was formed. In a 42 days' film the bacilli resembled serum bacilli, but a few longer forms were noted.

On Potato.—A thin layer of greyish growth was gradually formed. In a 56 days' film the bacilli varied in length from 1 to $4 \cdot 5\mu$. Many curved and many irregularly stained forms were noted.

SUMMARY.

Growth very poor, but better on potato than on glycerin-agar or broth.

(10.)

Strain.—CHIMPANZEE 4.

Material Received.—Third generation, 11 days old, of culture isolated from Chimpanzee 4.

On Dog and Bovine Serum.—The first culture, made on dog serum, produced a fairly good yield; the sub-culture from this on to bovine serum produced a good growth. On bovine serum, in a 16 days' film, the bacilli varied in length from $\cdot 75$ to $1 \cdot 5$ and occasionally 2μ. They were all uniformly stained, and, with the exception of a few which were slightly curved, were all straight.

On Broth.—Small speckled islands commenced to float about the middle of the third week, and the surface was nearly covered at the end of the fifth week. The pellicle then began to sink. The pellicle was thin, but slightly denser in patches, opaque, and had a rather moist appearance after the end of the first month. In a 26 days' film the bacilli varied in length from 1 to 2μ and were all straight and uniformly stained.

On Glycerin-Agar.—Growth very poor, no more than a fine grey haze being found at the end of nine weeks. In a 42 days' film the bacilli were very similar to those grown on bovine serum, but an occasional beaded form was noted.

On Potato.—A very slight increase was observed about the sixth week. In a 62 days' film many of the bacilli were indistinguishable from bacilli grown on serum; some of the bacilli were faintly stained or showed irregular thickenings; and there were some groups of bacilli which measured from 3 to 4μ in length; these last were curved and uniformly stained.

SUMMARY.

Growth poor. No better than the preceding strain.

(11.)

Strain.—CHIMPANZEE 6.

Material Received.—Primary culture, 23 days old, isolated from Chimpanzee 6.

On Dog and Bovine Serum.—The first sub-culture, made on dog serum, showed a well-marked increase in 6 days and a moderately good yield at the end of a fortnight. The first culture on bovine serum, made from the dog serum tube, produced a fairly good growth. Subsequent cultures were better. On dog serum, in a 14 days' film the bacilli varied in length from $\cdot 5$ to $1 \cdot 25\mu$, the average being less than 1μ. They were all straight and uniformly stained. On bovine serum, in a 20 days' film, the characters of the bacilli were identical with those grown on dog serum.

On Broth.—Delicate, translucent islands commenced to form during the third week. The surface was completely covered at about the end of the fifth week. The film was then very delicate, semi-translucent, and dotted with small grey points. No further thickening took place. There was no marked tendency to sink. In a 41 days' film the bacilli varied in length from $\cdot 75$ to 2μ, the great majority measuring from 1 to $1 \cdot 5\mu$. They were all straight and uniformly stained.

On Glycerin-Agar.—Growth very poor. A very thin, greyish layer covered two-thirds of the surface in eight weeks. In a 55 days' film the bacilli measured from 1 to $2 \cdot 5\mu$. They were almost all straight. With the exception of a few faintly stained forms they all stained well and uniformly.

On Potato.—There was no visible growth at the end of six weeks. Microscopic examination of a 41 days' film showed that, with the exception of some rather thicker forms, the characters of the bacilli were the same as those grown on serum.

SUMMARY.

Similar to the preceding strain.

(12.)

Strain.—CHIMPANZEE 8.

Material Received.—Second generation, 23 days old of culture isolated from Chimpanzee 8.

On Bovine Serum. The tube received only contained a scanty growth, and the tube inoculated from this gave a poor yield at the end of three weeks. Subsequent cultures grew well. In a 28 days' film nearly all the bacilli were straight and uniformly stained and varied in length from $\cdot 5$ to $1 \cdot 5\mu$. A few longer forms were found, measuring from 3 to 4μ; some of these were curved and some were regularly beaded.

On Broth.—Small, very delicate islands appeared at the beginning of the third week, and the surface was covered with a pellicle of the delicate, nearly translucent type at the end of the fifth week. In a 31 days' film the bacilli varied in length from 1 to $2 \cdot 5\mu$ and were straight and uniformly stained.

On Glycerin-Agar.—Growth extremely poor, only a faint grey haze being present at the end of eight weeks. In a 47 days' film the bacilli varied in length from 1 to 3μ; most of them were straight, many were beaded, and many contained globular swellings.

On Potato.—A very slight increase was observed during the first fortnight, but no growth took place after that period. In a 46 days' film the bacilli measured from 1 to 3μ; some were beaded and a few curved.

SUMMARY.

Growth very poor. Slightly inferior to the preceding strain.

(13.)

Strain.—CHIMPANZEE 10.

Material Received.—Primary culture, 42 days old, raised from a mesenteric gland of Chimpanzee 10.

On Horse Serum.—Growth good. In a 14 days' film forms as short as ·5μ were numerous ; the length of the bacilli on the whole varied from ·5 to 1·5μ, the average being under 1μ; they were straight and uniformly stained.

On Broth.—Growth was obtained with great difficulty. At the end of six weeks scattered patches of nearly transparent material covered about half the total surface. In a 56 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—No more than a fine grey haze was obtained at the end of two months. In a 42 days' film the bacilli resembled, on the whole, those grown on serum, but a few beaded forms were noted.

On Potato.—No visible growth was obtained at the end of two months. In a 60 days' film nothing was found but small clumps of bacilli which appeared to be portions of the material inoculated.

SUMMARY.

Growth very poor ; comparable to Chimpanzee 8.

(14.)

Strain.—CHIMPANZEE 16.

Material Received.—Primary culture, 19 days old, isolated from Chimpanzee 16.

On Horse Serum.—Growth poor. In an 18 days' film the bacilli varied in length from ·75 to 2μ. They were straight and uniformly stained.

On Broth.—Growth very poor. A few small, semi-transparent islands appeared in 5 weeks, and at the end of two months the surface was covered with a semi-translucent pellicle. In a 36 days' film the bacilli resembled those grown on serum, but were slightly longer.

On Glycerin-Agar.—A fine grey haze only was formed. In a 47 days' film the bacilli were only slightly longer than serum-grown bacilli. Many were faintly or irregularly stained.

On Potato.—No growth was obtained.

SUMMARY.

Growth very poor.

VIRUS—B V.

(Bovine Mesenteric Glands.)

(1.)

Strain.—RABBIT 11 from ORIGINAL MATERIAL.

Material Received.—Third generation, 8 days old, of culture isolated from Rabbit 11, which was inoculated with the original material.

On Horse Serum.—The first culture made was rather poor for the first fortnight, but then came on well. Subsequently cultures grew satisfactorily from the commencement. In an 18 days' film a few of the bacilli were curved, but the great majority were straight ; a few bacilli were regularly beaded and a few contained a darkly stained globular swelling. The bacilli varied in length from 2·5 to ·75μ, the average being 1μ.

On Bovine Serum.—Growth as on horse serum. In a 19 days' film the only difference noted from the horse serum growth was that forms containing a globular swelling were very rare.

On Dog Serum.—An abundant growth, becoming white and elevated during the third week. In a 14 days' film the bacilli were straight, uniformly stained, generally about ·75μ long, rarely longer than 1·25μ.

On Glycerinated Bovine Serum.—At the end of a week there was no uniform layer of growth, but pin-point colonies could be seen scattered all over the surface. At the end of a fortnight the surface was covered with a grey layer and there were several raised, white colonies. Steady increase took place during the third, fourth, and fifth weeks, and the ultimate yield was a thick, moist, whitish yellow growth with some discrete, elevated colonies. In a 21 days' film about one-third of the bacilli measured from ·75 to 1·5μ and were straight and uniformly stained. The remaining two-thirds, varied in length from 2·5 to 4·5μ ; most of them were curved ; a fairly large number were regularly beaded ; some had globular swellings ; and some were faintly stained.

On Broth.—Growth commenced at the end of the first week and the surface was covered at the end of the fourth week. The pellicle was semi-translucent on the whole, but contained several much denser, white patches. In a 27 days' film the bacilli exhibited great irregularity. At least half were curved, and about two-thirds were either regularly beaded or irregularly stained, the most conspicuous irregularities consisting of terminal thickenings and dark globular swellings. The length of the bacilli varied from over 5μ to 1·5μ, and the average was about 3μ.

On Glycerin-Agar.—A thin, opaque, grey layer covered the surface in the second week. Subsequent increase was slow but appreciable. Some rather dense grey colonies and patches were formed, but the general surface never became thickly covered ; the yield, at the end of two months, consisted of a thinnish grey layer beset with some much denser patches. In a 22 days' film the bacilli varied in length from 1·5 to 5μ ; the majority were curved ; many stained irregularly, and a moderate number were regularly beaded.

On Potato.—No increase was recognisable to the naked eye until the third week. A non-pigmented, thin layer of growth could then be recognised over most of the surface. This growth slowly increased, but never became very thick or heaped up. In a 25 days' film the bacilli varied greatly in size, shape, and staining properties. Length, from ·75 to 5 or 6μ, with every intermediate length about equally represented. Most of the longer forms were curved. Regularly beaded forms, irregular thickenings, and slender, faintly stained forms of every size were all numerous.

SUMMARY.

Growth good on serum ; rather slow, but ultimately good, on glycerinated serum ; on broth, glycerin-agar and potato by no means copious, but distinctly better than all the strains of Viruses B.I.–IV., with the exceptions of Dog 18 (B. IV.), Dog 92 (B IV.), and Baboon 8 (B.I.).

(2.)

Strain.—Goat 2.

Material Received.—Third generation, 29 days old, of culture isolated from Goat 2.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were all uniformly stained ; a few were curved. The bacilli varied in length from 2·5 to ·75μ, or slightly less, the average length being 1μ. In a 28 days' film of the same culture there was no morphological alteration.

On Bovine Serum.—Growth very good. Morphology of bacilli, in a fourteen days' film, as on horse serum.

On Dog Serum.—Abundant growth, forming a white, uniform layer in a fortnight and becoming heaped up at the end of a month. In a fourteen days' film the bacilli were all uniformly stained, almost all straight, and averaged slightly under 1μ in length.

On Glycerinated Bovine Serum.—At the end of a week the surface was well covered with a moderately thick, uniform growth ; on a control tube of the same bovine serum without glycerin the growth was fairly good, but perhaps slightly less. At the end of a fortnight there was a marked increase, the growth having become denser and greyer ; on pure serum the growth was, perhaps, rather less. Small white elevations appeared during the third week. Rapid increase occurred during the fourth and fifth weeks. The final yield was a dense, white, moist growth, distinctly more copious than the growth on pure serum. In a fifteen days' film the bacilli measured from 2 to 7μ and averaged about 4μ. Most of the bacilli were curved. Some of them were regularly beaded, but at least three-fourths of them contained globular thickenings. From one to six or seven of these thickenings were found in a single bacillus.

On Broth.—Patches of fairly thick pellicle began to appear at the end of the first week. The pellicle then steadily increased and covered the surface in from four to five weeks. It then became moist at the margins and began to sink. The pellicle, though fairly thick on the whole, was unequal in density ; the thicker portions, as they extended, had a tendency to thin out into material which was barely thick enough to be opaque. In a twenty-one days' film the bacilli exhibited great irregularity. Curved forms were quite as numerous as straight, and the majority of the bacilli were either beaded, or exhibited irregular thickenings, or contained globular swellings. The length of the bacilli varied from 6μ to 1·5μ, the average being over 3μ.

On Glycerin-Agar.—The entire surface became covered with a thin, opaque growth during the second week. This growth steadily increased during the three following weeks ; it exhibited many small, raised, grey colonies and was characterised by a rather moist appearance. After the fifth week very little development took place, and the ultimate yield consisted of a grey layer of unequal density, consisting of dense patches and colonies situated amongst much thinner material. In a twenty-one days' film the bacilli exhibited great irregularity of staining. Forms with irregular thickenings or globular swellings and faintly stained forms were very numerous. About half the bacilli were curved, and many of them exhibited tapering ends. The length of the bacilli varied from 1 to 5μ.

On Potato.—Minute grey colonies were observed at the beginning of the second week. These made very little appreciable increase until the fourth week, when they became raised and much larger. The colonies did not become heaped up, but between individual colonies a thin layer of growth was formed. The growth was not pigmented. In a twenty-six days' film there were a fair number of short, straight, uniformly stained bacilli, measuring from 1 to 1·5μ ; a much larger number of the organisms present were curved, from 3 to 5μ in length, faintly and unequally stained, or in a few cases, regularly beaded.

Summary.

Growth perhaps rather better than Rabbit 11.

(3.)

Strain.—Heifer 80.

Material Received.—Twenty-third generation, 6 days old, of culture isolated from Heifer 80.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 1·5μ and were straight and uniformly stained.

On Broth.—In 10 days the surface was covered and the pellicle was spreading on to the glass. It was thin, grey, and uniformly opaque. At the end of three weeks the pellicle had become moist and fallen to the bottom of the flask. In a 21 days' film the bacilli varied in length from 1 to 3·5 and occasionally 4μ. Most of them were straight. Irregularly stained forms were present.

On Glycerin-Agar.—A thin, opaque, grey layer, denser in patches, was formed in 10 days. At the end of three weeks the layer was thicker and the general surface was fairly well covered. Very little further increase took place, the total yield consisting of a grey layer of unequal density, and being on the whole rather scanty. In a 42 days' film the bacilli varied in length from ·75 to 3·5μ. Many of them were curved. The staining was very irregular, discrete beading, globular thickenings and other irregularities all being common.

On Potato.—Visible growth commenced during the second week, and produced scanty, grey patches in the third week. Very little increase took place subsequently. In a 56 days' film the bacilli varied in length from 1 to 3.5μ. The majority were straight. Many groups stained irregularly.

Summary.

Growth not quite so good as with Goat 2.

(4.)

Strain.—Dog 80 from Original Material.

Material Received.—Second generation, 19 days old, of culture isolated from the liver of Dog 80.

On Horse Serum.—Growth satisfactory ; after some generations of subculture, good. In an 18 days' film the bacilli varied in length from ·5 to 1·5 or 2μ. They were straight and uniformly stained.

On Broth.—Growth was slow during the first three weeks. After that period the surface rapidly became covered with an opaque, thin, uniform pellicle, which became moist and sank during the sixth week. In a 38 days' film the bacilli resembled those grown on serum, but were rather longer.

On Glycerin-Agar.—Growth slow but steadily maintained for about 5 weeks. The surface became well covered and had a granular appearance, due to the aggregation of discrete, slightly raised colonies. In a 42 days' film the bacilli varied in length from ·75 to 3·5μ, occasionally 4 to 5μ. Curved forms were frequent Beading or irregular staining were not common.

On Potato.—A scanty, yellowish growth was slowly formed. In a 56 days' film the bacilli varied in length from ·75 to 3μ, occasionally 4μ. They were for the most part uniformly stained, and, except the longer forms, straight.

Summary.

Growth, on the whole, no better than with other strains of this virus.

VIRUS—B VI.

(Bovine Mediastinal Glands.)

(1.)

Strain.—Original Material.

Material Received.—Second generation, thirty-seven days old, of culture isolated from original material.

On Horse Serum.—The first culture developed rather poorly, the surface gradually becoming covered with a fine, thin layer of growth, dotted over with grey colonies of moderate size. Subsequent cultures gave a moderately good yield. In a twenty-four days' film, the bacilli were all uniformly stained, almost all straight, from ·5 to 1·5μ (occasionally 2μ) in length; and averaged about ·9μ.

On Bovine Serum.—With the exception of the first culture, which was poor, the growths were satisfactory. In a twenty-four days' film the bacilli differed very little from those grown on horse serum, but an occasional form as long as 3μ was noted.

On Dog Serum.—The surface was covered with a dull, grey, uniform layer in seven days. In fourteen days the growth was increased, but was still perfectly uniform. In three weeks greyish elevated streaks and white colonies appeared. Some further increase was observed at the end of the fourth week. In a fifteen days' film the bacilli were identical with those grown on horse serum.

On Glycerinated Bovine Serum.—At the end of a week there was hardly any growth; on the control tube of the same bovine serum without glycerin the surface was covered. At the end of a fortnight there was a slight but definite increase; the growth on pure serum was better. At the end of three weeks there was a considerable increase, the growth being rather thin but well distributed and containing grey colonies; the growth on pure serum was little, if any, better. At the end of a month the grey colonies had increased, but there was no increase on the rest of the surface; on pure serum the total amount of growth was no greater but it was uniformly distributed, instead of consisting mainly of aggregations of discrete colonies. There was perhaps a little further increase during the fifth week. In a twenty-one days' film the bacilli measured from ·75 to 2·5 and occasionally 3μ. A few were curved, a very occasional bacillus was regularly beaded; the rest were uniformly stained.

On Broth.—No growth appeared on the broth before the fifth week. A pellicle then began to form and the surface was completely covered in two months. Two broth cultures were made, on samples of the same broth. The rates of growth were about the same in the two cases, but the types of pellicle differed. In one of them the pellicle was of the thin semi-transparent type, speckled with white circular spots and small irregular islands; in the other, the pellicle was uniformly grey and opaque, with no raised colonies or islands, and though very thin on the whole, was of unequal density. In a 50 days' film from the one broth and a 72 days' film from the other the bacilli were identical in characters. They measured from 1 to 2·5μ, were occasionally curved, and were all uniformily stained.

On Glycerin-Agar.—Growth extremely slow and scanty. At the end of two months there was a very thin greyish film in which a few minutes dense points were distributed. In a 77 days' film the great majority of the bacilli were straight, uniformly stained, and measured from ·5 to 1·25μ. A few bacilli were about 2μ long; these were straight and regularly beaded.

On Potato.—Growth very poor, but at the end of two months a decided, though very scanty, increase was obtained. The growth resembled the potato in colour and was most readily recognised on removal with the platinum loop. In a 63 days' film most of the bacilli measured from 1·5 to 2·5μ; occasional forms were longer. Nearly all were straight and uniformly stained. There were a few globular swellings, but no regularly beaded forms.

Summary.

Growth poor on all media except serum and very poor on glycerin-agar.

(2.)

Strain—Calf 240.

Material Received.—Third generation, 39 days old, of culture isolated from Calf 240.

On Bovine Serum.—Growth good. In a 12 days' film the bacilli varied in length from ·75 to 1·5 or 2 μ. They were straight and uniformly stained.

On Broth.—Growth commenced in the third week and the surface was nearly covered at the end of eight weeks. The pellicle was thin and semi-transparent on the whole, but contained denser patches which exhibited a tendency to sink. In a 53 days' film the bacilli varied in length from ·75 to 3 μ. The longer forms were sometimes curved, but rarely beaded.

On Glycerin-Agar.—A very thin, greyish layer was formed at the end of a fortnight. Not much further change was noted until the seventh week, when the growth became rather denser and assumed a fine, broken-glass appearance. In a 43 days' film the bacilli resembled those grown on serum, with the exception of a few irregularly and faintly stained forms.

On Potato.—A few grey colonies appeared in the fourth week and then slowly increased and became slightly yellow. In a 51 days' film the bacilli resembled serum bacilli, with the exception of occasional longer forms.

Summary.

Growth rather better than strain from original material.

VIRUS—B VII.

(Bovine Mesenteric Glands.)

Strain.—G. P. 1017 from Original Material.

Material Received.—Fourth generation, 33 days old, of culture isolated from G. P. 1017.

On Horse Serum.—Growth satisfactory. In a 20 days' film most of the bacilli measured from ·75 to 1μ; a few forms were found from 1·5 to 2μ. The bacilli were straight and uniformly stained.

On Bovine Serum.—Growth as on horse serum. Morphology, in a 21 days' film, as on horse serum, but possibly a little shorter.

On Dog Serum.—The growth commenced as a uniform grey layer, which became dense at the end of the second week; between the third and fourth weeks it exhibited white, raised colonies and patches. In an 18 days' film the bacilli resembled those grown on horse serum.

On Glycerinated Bovine Serum.—At the end of a week several minute, discrete colonies were appearing, but there was very little growth on the rest of the surface; on the same serum without the addition of glycerin

the surface was moderately well covered. At the end of a fortnight there was a moderately thin film of semi-translucent growth; there was a moderate growth on pure serum. At the end of three weeks there was a marked increase, the general layer of growth being thicker and the grey colonies more definite; the amount of growth on pure serum was about the same. At the end of a month the small, discrete, grey colonies were more numerous and were closely set together; the growth on pure serum was rather better. No change occurred after the fourth week, the total yield being poor, but not very poor. In a 21 days' film about two-thirds of the bacilli were straight, uniformly stained and measured from 1 to 2μ; the rest measured from 2 to 4·5μ; they were often regularly beaded and often contained globular swellings.

On Broth.—A pellicle began to form during the second week. The surface was two-thirds covered at the end of the third week and completely covered at the end of the fourth week. The pellicle was opaque and thin but of slightly unequal density; it readily became moist and sank. In a 22 days' film the bacilli measured from 1 to 4μ, averaging about 3μ. Of the bacilli measuring over 2μ more than half were curved. About one-third of the bacilli were beaded, some regularly and others irregularly. There were also a fair number of faintly stained forms, some with globular swellings.

On Glycerin-Agar.—At the end of three weeks the surface had become greyish and exhibited several dense points. Subsequent increase was very slow. At the end of two months there were many grey colonies, rather larger than pin points. In a 51 days' film the bacilli measured from 1 to 3·5 or 4μ. The shorter forms were straight, uniformly stained, and sometimes rather thick. The longer forms were frequently curved; some of them were regularly beaded and some contained a globular thickening.

On Potato.—A slight increase was noted in a fortnight and many small grey colonies were visible at the end of a month. In two months' time, there was, in addition to these colonies, a slight layer of growth over most of the surface. In a 38 and in a 51 days' film the characters of the bacilli were the same. They varied in length from ·5 to 4·5μ. The longer forms, which were more numerous than the short, were nearly all curved. Some of them were regularly beaded and many contained a very conspicuous oval thickening which was situated sometimes in the middle and sometimes at one end of the bacillus. Many bacilli were thicker than usual; these were stained uniformly but were often of somewhat unequal thickness in different portions of their length. Bacilli were frequently found in pairs, the one bacillus being in close juxtaposition to the other throughout its length; sometimes one of these bacilli was much shorter than the other. Sometimes groups of three or four bacilli appeared to be attached to each other at one extremity. Occasionally the thick, irregularly shaped bacilli appeared to be surrounded by a halo suggestive of a capsule.

SUMMARY.

Growth on serum and glycerinated serum moderately good; on both growth rapid, with a tendency to become moist and sink; on glycerin-agar and potato rather poor.

VIRUS—B VIII.

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 37 days old, of culture isolated from original material.

On Horse Serum.—The first culture developed rather slowly for the first fortnight, but at the end of a month yielded a fairly good growth, with raised colonies of considerable size. Subsequent cultures grew more readily. In a 12 days' film the bacilli were all uniformly stained, nearly all straight, and varied in length from ·5 to 2μ, the majority being about 1μ.

On Bovine Serum.—The first culture made grew more rapidly and abundantly than the corresponding culture on horse serum. Subsequent growths were good. In a 14 days' film the morphological characters were identical with those found on horse serum.

On Dog Serum.—The surface was well covered with a thick, uniform, grey growth in 7 days. In a fortnight there was a considerable increase, the growth becoming raised and white in patches. Further increase took place during the third and fourth weeks, and elevated white colonies were observed. In a 15 days' film the bacilli were all uniformly stained and almost all straight; they measured from ·75 to 1·25μ, the average being not much over ·75μ.

On Glycerinated Bovine Serum.—A thin layer of growth was formed at the end of the first week. This steadily increased until the end of the fourth week and then remained stationary. The growth was moderate in amount, grey, uniformly distributed, with no discrete colonies, and very moist. In a 21 days' film about one-fourth of the bacilli were uniformly stained, straight, and about 1μ long. The remainder varied in length from 2·5 to 4·5μ; most of them were curved; a very large number were regularly beaded; some contained globular swellings, and some were faintly stained.

On Broth.—The bacilli commenced to grow from the serum on to the surface of the broth shortly before the end of the first fortnight. The pellicle then advanced steadily, uniformly, and rather slowly, the greater part of the surface being covered in about 5 weeks. The pellicle formed was continuous, grey, homogeneous, and moderately dense. After about 5 weeks the pellicle began to break up and sink. In a 37 days' film the bacilli bore a striking resemblance to Kossel's description of the "bovine type." There were (1) short, straight, homogeneously stained, rather thick forms measuring from ·75 to 1·25μ; (2) straight forms from 1·5 to 2·5μ in length, which were sometimes unequally stained; (3) forms from 2·5 to 4·5μ long, mostly curved and very rarely homogeneously stained; of these some were regularly beaded, some had globular swellings, some exhibited a thickened extremity, and some contained deeply stained granules and were "suggestive of diphtheria bacilli"; (4) many faintly stained forms, curved and measuring from 3 to 4·5μ.

On Glycerin-Agar.—There was a thin film of greyish growth at the end of a fortnight; at the end of the third week there was a decided increase, many punctiform, definitely grey colonies making their appearance, particularly near the margins of the surface; after this period extremely little further growth took place until the end of the second month; further increase then ensued, and in 90 days the surface was covered with fairly large, discrete, elevated, somewhat pear-shaped, greyish-yellow colonies. A control tube behaved in the same manner. In a 34 days' film the bacilli exhibited a marked pleomorphism. Irregularly beaded forms and forms with globular swellings were very abundant. Many bacilli stained somewhat faintly. Considerably more than half the bacilli were curved. Though some shorter forms occurred, the length of the majority varied from 2·5 to 6μ, a good many being over 4μ.

On Potato.—Some increase was observed at the end of the first fortnight, but this was not maintained, and a very scanty grey growth was all that resulted at the end of two months' incubation. During the third month discrete grey colonies appeared, and increased in size. In a 60 days' film, amongst short, straight, uniformly stained forms (1 to 2μ) were found a smaller number of curved beaded forms, measuring from 4 to 6μ, some of them with globular swellings.

SUMMARY.

Growth similar to that of B VII.

VIRUS—B IX.

(Bovine Mediastinal Gland.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 41 days old, of culture isolated from original material.

On Horse Serum.—Good growth. In a 26 days' film the bacilli were straight and uniformly stained, varied in length from ·5 to 1·25μ, and averaged about ·75μ.

On Bovine Serum.—As on horse serum. In a 26 days' film the bacilli resembled those grown on horse serum.

On Dog Serum.—Grew rapidly and abundantly, being dull grey and uniform until the third week, when raised white colonies appeared. In a 15 days' film most of the bacilli measured from ·75 to 1·25μ ; a few were from 1·5 to 2μ ; the average was rather under 1μ. They were straight and uniformly stained.

On Glycerinated Bovine Serum.—At the end of a week there was an abundant growth, becoming raised and white in patches ; a culture on the same serum, to which no glycerin had been added, was equally good. At the end of a fortnight the growth had increased considerably in thickness ; the pure serum culture, though good, was less abundant. During the third and fourth weeks a vigorous increase was maintained ; the pure serum culture showed very little change and was much less abundant. No increase was observed after the fourth week. The final yield was a moist, copious, yellowish, irregularly heaped up growth. In a 21 days' film an occasional bacillus was short (about 1μ), straight, and uniformly stained ; the rest measured from 2·5 to 4·5μ, and were nearly all curved, very often regularly beaded, sometimes with globular swellings and sometimes faintly stained.

On Broth.—Grew very rapidly. The surface was more than half covered in 12 days and was completely covered at the end of three weeks. The pellicle was opaque, dense, and somewhat heaped up in patches. It very readily became moist and sank. A second flask which was inoculated exhibited the same characteristics. In a 34 days' film the bacilli measured from 1 to 2·5μ ; a few were curved ; with the exception of an occasional beaded form, all were uniformly stained.

On Glycerin-Agar.—Growth fairly good. At the end of three weeks the surface was moderately well covered with a thin layer which had begun to assume a granular, frosted-glass appearance. Raised colonies began to appear during the fourth week, and the culture continued to increase slowly until the end of the second month. It was then fairly abundant, of the frosted glass type, beset with numerous grey, polypoid colonies, and without any wrinkling. In a 55 days' film the bacilli measured from 1 to 3μ. They were nearly all straight or only slightly curved. A few of the larger forms were regularly beaded ; some contained globular swellings, and some were faintly stained.

On Potato.—Small grey colonies appeared during the second week. During the third and fourth weeks the growth increased ; the colonies become more raised and grey patches appeared on the rest of the surface. In a 36 days' film the bacilli measured from 1·5 to 4·5μ. The shortest forms were not very numerous ; they were straight and uniformly stained. The rest were all stained either unequally or faintly ; a good many of them were regularly beaded and a moderate number contained globular swellings ; about half of them were curved.

SUMMARY.

Growth more luxuriant than with any strains of the preceding viruses except Dog 18 (B IV.) and Dog 92 (B IV.).

(2.)

Strain.—BABOON 68.

Material Received.—Primary culture, on egg, of material isolated from Baboon 68.

On Horse Serum.—Growth was poor until the third generation ; afterwards it was satisfactory. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ and averaged about ·75μ ; they were straight and uniformly stained.

On Broth.—The surface in one flask was half covered at the end of a fortnight with an opaque, uniform, moderately thick layer. In another flask the surface was completely covered, in the same period, with a pellicle of the same type. In both flasks the pellicle became moist during the third week and eventually all fell to the bottom. In a 21 days' film the bacilli varied in length from ·75 to 3μ. The majority were straight. The staining was very irregular.

On Glycerin-Agar.—A fairly dense, grey layer with some raised patches was obtained during the second week. During the third week there was a marked increase, which was maintained slowly until the end of the fifth week. The total yield was a fairly dense but unequally distributed grey layer with a moist appearance. In a 41 days' film, the bacilli varied in length from ·75 to 2·5μ. Most of them were straight ; their staining was irregular.

On Potato.—A somewhat thick grey layer was produced in a fortnight. This increased until about the sixth week and produced many raised colonies. In a 56 days' film the bacilli varied in length from ·75 to 4·5μ. The majority were straight and uniformly stained ; but a considerable number were curved and stained irregularly.

SUMMARY.

Growth similar to the strain of B IX. isolated from the original material.

(3.)

Strain.—CAT 52 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 6 days old, of culture isolated from Cat 52 (a kitten).

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·5μ or ·75 to 1·5μ, and averaged less than 1μ.

On Broth.—The surface was almost covered in 10 days with a thin, grey, uniform pellicle. This pellicle appeared rather thickened during the third week, but soon became moist, and, during the fourth week, all fell to the bottom. In a 21 days' film the bacilli varied in length from 1 to 3μ. They were, for the most part, uniformly stained. Many of the longer forms were curved.

On Glycerin-Agar.—A grey, moderately dense layer was found in 10 days. This increased until about the fifth week, and then growth ceased. The growth showed several dense, moist, granular patches, but the rest of the

layer was thin. In a 42 days' film the bacilli varied in length from ·75 to 3·5 and occasionally 4·5μ. A large number were curved. The staining was very irregular.

On Potato.—Some increase was observed during the second week, and in the fourth week there were many grey colonies and patches. At the end of two months, in addition to these colonies, a grey layer was visible over the whole of the surface. In a 56 days' film the bacilli varied in length from 1 or 1·5 to 4 or 4·5μ. They were slender, curved, and stained irregularly. Globular swellings were numerous.

SUMMARY.

Growth on the whole slightly inferior to the strain of B IX from the original material.

(4.)

Strain.—CHIMPANZEE 14.

Material Received.—Primary culture, 35 days old, isolated from an ileo-colic gland of Chimpanzee 14.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were rather unusually small. They varied in length from about ·5 to about 1·25μ, and averaged not more than ·75μ. They were straight and uniformly stained.

On Broth.—The surface was two-thirds covered with an opaque, uniform, not very dense pellicle in about a fortnight. The pellicle then became moist and fell to the bottom of the flask. In a 19 days' film the bacilli varied in length from ·75 to 3·5μ. The majority were straight; globular swellings were numerous.

On Glycerin-Agar.—A fairly abundant grey layer, with thickened patches, was formed in a fortnight. This gradually increased, the ultimate yield being a moderately thick, rather moist growth. In a 42 days' film the bacilli, for the most part, varied in length from 1 to 4μ. There were also some small, coccal forms, measuring from ·5 to ·75μ. The longer bacilli were generally curved and irregularly strained; sometimes they were regularly beaded. Globular swellings were very numerous.

On Potato.—Grey colonies and patches were seen at the commencement of the third week, and at the end of five weeks there was a fairly abundant, grey growth. In a 56 days' film the bacilli varied in length from ·75 to 5μ. The majority of the longer forms were curved. Staining was very irregular.

SUMMARY.

Growth not quite so good as the strain of B IX from the original material.

(5.)

Strain.—G. P. 1708 from DOG 50.

Material Received.—Fourth generation, 17 days old, of culture isolated from G. P. 1708, which was inoculated from Dog 50.

On Horse Serum.—Growth good, the great majority of the bacilli varied in length from ·5 to 1·5μ; they were rarely as long as 2μ. The bacilli were straight and uniformly stained.

On Broth.—The first culture made rapidly formed a fairly thick grey pellicle which showed some tendency to sink when it became a month old. Later cultures showed no tendency to sink. They yielded a thick grey pellicle, which covered the surface in a month and then grew on to the glass. The pellicle, though decidedly thick and tough, was not uniformly thick, and was not of the densely wrinkled type. In a 22 days' film the bacilli varied in length from 1 to 4μ. About half were curved and about one-third were beaded.

On Glycerin-Agar.—A dense, prominently wrinkled layer was formed during the first fortnight and continued to increase during the three following weeks. In a 42 days' film the bacilli varied in length from 1 to 4·5μ. Curved forms were very frequent. There was a tendency to regular beading; some globular swellings were also noted.

On Potato.—A brown layer of growth appeared in a fortnight. It gradually increased in amount, and became raised in patches. In a 56 days' film the bacilli varied in length from 1 to 5μ, or longer. They were mostly curved and exhibited a tendency to beading. Many globular thickenings were noted.

SUMMARY.

Compared with the strain of B IX from the original material, there is a very marked increase in luxuriance. This strain grows more abundantly than Dog 18 (B IV.).

(6.)

Strain.—DOG 60.

Material Received.—Third generation, 17 days old, of culture isolated from Dog 60 (a pup).

On Bovine Serum.—Growth good. In a 14 days' film, the bacilli varied in length from ·5 to 1.5μ. The average, was not over ·75μ; forms as short as ·5μ were numerous.

On Broth.—At the end of a fortnight the surface was two-thirds covered with a rather thin, opaque, uniform pellicle. At the end of the third week the surface was completely covered; the pellicle was rather thicker and was becoming moist. At the end of a month, most of the pellicle had fallen to the bottom of the flask. In a 21 days' film the bacilli varied in length from about 1 to about 3·5μ. They were straight, with the exception of some of the longer forms. Staining was very irregular.

On Glycerin-Agar.—A well marked grey layer was formed at the end of the first week. This was much denser at the end of a fortnight. Growth steadily increased until the end of the fifth week. The ultimate yield was thick, irregularly distributed, moist, and partly wrinkled. In a 42 days' film the bacilli varied in length from 1 to 3·5 or 4μ. The great majority were curved and stained irregularly.

On Potato.—Raised, slightly yellow colonies were formed in a fortnight. The growth increased until about the sixth week but did not become abundant. In a 60 days' film the bacilli were straight and uniformly stained and about the same length as those grown on serum.

SUMMARY.

The cultures are of the same character as those obtained from the strain isolated from the original material, but on the whole, are rather more luxuriant.

(7.)

Strain.—Dog 84 from Dog 60.

Material Received.—Second generation, 19 days old, of culture isolated from the lung of Dog 84.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from ·5 to 1·5μ, and were straight and uniformly stained.

On Broth.—Growth uncertain. In the first flask inoculated the surface was covered in a fortnight with a pellicle which rapidly became moist and sank. In another flask growth was much slower, and the pellicle was of a tougher and denser type, showing no tendency to sink. In a 38 days' film the bacilli varied in length from 1 to 3·5μ. They were uniformly stained. A few of the longer forms were curved.

On Glycerin-Agar.—Growth was maintained continuously but slowly during the first five weeks. The surface was then covered with a moderately thick layer which had a granular appearance, owing to the presence of innumerable small, raised colonies. In a 42 days' film the bacilli varied in length from 1 to 5 or 6μ; curved forms were frequent. More than half were uniformly stained, but regularly beaded and irregular forms were fairly numerous.

On Potato.—A fairly abundant, grey, crumb-like layer was slowly formed. In a 56 days' film small forms (·5 to ·75μ) were very numerous. The rest of the bacilli varied in length up to 4·5μ and were generally straight and irregularly stained.

SUMMARY.

One of the better growing strains of B IX.

(8.)

Strain.—Goat 32.

Material Received.—Fourth generation, 6 days old, of culture isolated from Goat 32.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ, averaging slightly under 1μ. They were straight and uniformly stained.

On Broth.—The surface was covered in 10 days with a thin, opaque, uniform layer. The pellicle then became moist, and had all fallen to the bottom of the flask soon after the beginning of the fourth week. In a 20 days' film the bacilli varied in length from 1 to 3μ. They were straight, or only slightly curved, and uniformly stained.

On Glycerin-Agar.—A grey, moderately thick layer was formed in 10 days and further increase took place until about the end of the fifth week. The total yield was moderately dense, moist, and with raised colonies and patches. In a 42 days' film the bacilli varied in length from ·75 to 4 or 4·5μ. Straight forms were much commoner than curved. The staining was very irregular.

On Potato.—Definite increase was observed during the second week, and at the end of a month there were many grey colonies and patches, the intervening surface being covered with a thin, uniform layer. In a 56 days' film the bacilli varied in length from 1 or 1·5 to 4 or 4·5μ. Curved forms were frequent; some of these were notably slender. The staining was highly irregular.

SUMMARY.

Growth perhaps slightly poorer than that of the strain of B IX isolated from the original material.

(9.)

Strain.—Hedgehog 22.

Material Received.—Fourth generation, 17 days old, of culture isolated from Hedgehog 22.

On Bovine Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ; a few forms measured from 2 to 3μ. The bacilli were uniformly stained. A few were curved.

On Broth.—The surface was covered with a moderately dense, uniform, grey layer in about a fortnight. After this period the pellicle became moist and began to sink. In a 22 days' film the bacilli varied in length from 1 to 4·5μ. The longer forms were curved. About two-thirds were irregularly stained.

On Glycerin-Agar—A well marked grey layer was formed at the end of a week. This increased rapidly until the end of the third week, but not much change was noted afterwards. The total yield was a grey, irregularly distributed growth with many raised colonies and patches. In a 42 days' film the bacilli varied in length from ·75 to 3·5μ. More than half were straight and uniformly stained. Many of the longer bacilli were curved. Globular swellings and forms with thickened extremities were found.

On Potato.—Numerous white colonies were found all over the surface at the end of a fortnight. Slow increase continued after this period, the total yield at the end of two months being fairly good. In a 61 days' film the bacilli exhibited great variety. They varied in length from ·5 to 5μ. Most of the longer forms were curved. The staining was highly irregular.

SUMMARY.

About equal to B IX from original material.

(10.)

Strain.—Lemur 10.

Material Received.—Primary culture, on egg, 26 days old, isolated from Lemur 10.

On Horse Serum.—Poor for the first two or three generations; afterwards satisfactory. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ. They were straight and uniformly stained.

On Broth.—The surface was covered in about a fortnight with an opaque, rather thin, homogeneous pellicle. During the third and fourth weeks the pellicle became moist and sank. In a 21 days' film the bacilli varied in length from 1 to 3 . The majority were straight, some groups of bacilli were stained uniformly; others exhibited numerous globular swellings.

On Glycerin-Agar.—A thin but opaque layer covered the surface in 12 days. The growth subsequently thickened, becoming moderately dense at the end of five weeks. The growth had a rather moist appearance. In a 41 days' film the bacilli varied in length from ·75 to 3μ. The longer forms were curved. The staining was very irregular.

On Potato.—A definite grey layer was formed in a fortnight. Some increase took place afterwards, but the growth never became abundant. In a 56 days' film the bacilli varied in length from ·75 to 4·5 or 5μ. The shorter forms were straight and uniformly stained; the longer were curved and were either regularly beaded or exhibited globular thickenings.

SUMMARY.

Growth on the whole slightly inferior to B IX. from original material.

(11.)

Strain.—MONGOOSE 14.

Material Received.—Fourth generation, 6 days old, of culture isolated from Mongoose 14.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ, averaging rather less than 1μ.

On Broth.—The surface was completely covered in 10 days with a thin, grey, uniform layer which was also spreading on to the glass. Very little thickening took place. The pellicle soon became moist, and soon after the commencement of the fourth week nearly all of it had fallen to the bottom of the flask. In a 21 days' film the bacilli varied in length from 1 to 4μ. Most of them were straight. The staining was highly irregular.

On Glycerin-Agar.—A grey layer, with many thickened points, appeared during the second week. During the third week there was a well-marked increase, and the growth assumed a frosted appearance. At the end of two months the total yield was moderately dense, but irregularly distributed, with raised grey colonies and patches. In a 42 days' film the bacilli varied in length from ·75 to 3.5μ. Many of the longer forms were curved. The staining was highly irregular.

On Potato.—Growth appeared during the second week. At the end of the second month the surface was moderately well covered with a grey layer, which exhibited many raised grey colonies. In a 56 days' film the bacilli varied in length from 1 or 1·5μ to 4 or 4·5μ. Many of them were notably slender; curved forms were frequent. The staining was highly irregular.

SUMMARY.

Growth slightly inferior to B IX. from original material.

(12.)

Strain.—MONKEY 116.

Material Received.—Primary culture, on egg, 26 days old, isolated from Monkey 116.

On Horse Serum.—Growth poor until the third generation; afterwards satisfactory. In a 14 days' film the bacilli varied in length from ·75 to 1·5μ. They were straight and uniformly stained, with the exception of a few forms which exhibited globular swellings.

On Broth.—The surface was covered in three weeks with a rather thin, opaque, uniform pellicle. The pellicle soon after this period became moist and sank to the bottom of the flask. In a 21 days' film the bacilli varied in length from 1 to 3μ. They were straight, or only slightly curved, and stained uniformly.

On Glycerin-Agar.—A scanty, grey layer was formed during the first fortnight. This increased very considerably during the three following weeks. The total yield was a moist, grey layer, with several thick, raised patches. In a 41 days' film the bacilli, with the exception of a few rather longer forms, resembled those grown on serum.

On Potato.—A grey, rather scanty growth. In a 59 days' film the bacilli varied in length from ·75 to 3·5μ. The great majority were straight and stained uniformly, but a small number of irregularly stained forms occurred.

SUMMARY.

Growth not so good as that of B IX from the original material. The bacilli grown on the various test media are notably straight and uniformly stained. These characters are exceptional with strains of the B IX virus.

(13.)

Strain.—PIG 108.

Material Received.—Fourth generation, 6 days old, of culture isolated from Pig 108.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ. They were straight and uniformly stained, with the exception of a few which exhibited globular swellings.

On Broth.—In 10 days the surface was covered with a thin, grey, uniform pellicle, which was also growing on to the glass. Before the completion of the fourth week all the pellicle had become moist and fallen to the bottom of the flask. In a 21 days' film the bacilli varied in length from 1 to 3·5μ. The longer forms had a tendency to be curved and stained irregularly, but the majority of the bacilli were straight and uniformly stained.

On Glycerin-Agar.—A fairly dense grey layer was formed in 10 days. Further increase took place until about the end of the fifth week. The total yield was moist, grey, with raised patches of dense material, between which the growth was much thinner. In a 42 days' film the bacilli varied in length from ·75 to 3μ. The majority were straight. The staining was irregular.

On Potato.—A well marked growth appeared during the second week. The total yield was moderately abundant. There was no pigment formation. In a 56 days' film the bacilli varied in length from 1 to 6μ, being longer than usual with this virus. Most of them were curved. Regularly beaded forms and also forms with globular swellings were very numerous.

SUMMARY.

Slightly inferior to the strain of B IX from the original material.

(14.)

Strain.—RABBIT 347 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 6 days old, of culture isolated from Rabbit 347.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ, and averaged about ·75μ. They were straight and uniformly stained.

On Broth.—In 10 days the surface was covered with a thin, grey, uniform pellicle, which was also creeping on to the glass. The pellicle did not grow any denser, but soon became moist. It had all fallen to the bottom of the flask by the end of the fourth week. In a 21 days' film the bacilli varied in length from ·5 to 2μ, being no longer than those grown on serum; coccal forms, and short, thick, oval forms were numerous. Very few bacilli were curved. There were some faintly stained and some irregularly stained forms.

On Glycerin-Agar.—The growth presented a grey, frosted glass appearance towards the end of the second week, but did not make much progress subsequently, no

more than a scanty grey layer being obtained at the end of two months. In a 42 days' film the bacilli varied in length from ·75 to 3·5μ. Many of the longer forms were curved and regularly beaded. Globular swellings were present but not numerous. On the whole, the irregularity of staining was not great.

On Potato.—Small grey colonies and patches were definitely visible in the third week. At the end of two months these areas had increased, and the rest of the surface was covered with a thin grey layer. In a 56 days' film the bacilli varied in length from 1 or 1·5 to 4μ. Many of them were curved. The staining was irregular.

SUMMARY.

Growth slightly poorer than any other strain of B IX.

VIRUS—B X.

(Bovine Mesenteric Glands.)

Strain—G.P. 1070 from ORIGINAL MATERIAL.

Material Received.—Fifth generation, 14 days old, of culture isolated from G.P. 1070.

On Horse Serum.—Good growth. In a 20 days' film the bacilli measured from ·75 to 1·5μ, the average being rather under 1μ. They were straight and uniformly stained.

On Bovine Serum.—Good growth. Morphology, in an 18 days' film, as on horse serum.

On Glycerinated Bovine Serum.—At the end of a week there was a copious grey growth with many small, raised, white colonies. There was some increase during the second week and at the end of the third week there was a thick growth of the ground glass type, with raised white colonies and patches. The growth increased considerably during the fourth week, and the ultimate yield was a dull, dense, white growth, more abundant than any of the cultures previously described, except B. IX. In a 35 days' film the bacilli were of two types:—(1) straight, uniformly stained forms from 1 to 2μ long; (2) forms from 3 to 7μ long which were frequently curved, irregularly stained, and sometimes regularly beaded.

On Broth.—A thick pellicle began to stream off the serum at the end of the second week. At the end of the third week the surface was half covered. During the fourth week the surface became almost completely covered; the pellicle then became moist and sank and no further surface growth was obtained. The pellicle was grey, fairly dense, and somewhat heaped up in patches. In a 31 days' film the bacilli measured from ·75 to 3μ and were nearly all straight. About half were stained uniformly and deeply; the rest were stained unequally, often faintly, and were sometimes beaded. Many of the bacilli were unusually thick.

On Glycerin-Agar.—At the end of a fortnight the surface was well covered with a moist, beaded, semi-translucent growth; this, at the end of the third week, had become converted into a mass of pin-point grey colonies. The growth continued to increase slowly and assumed the frosted-glass type; it never became very copious. In a 31 days' film the bacilli measured from 1 to 3·5μ. Many of the longer forms were slightly curved and irregularly beaded; some of them had tapering ends.

On Potato.—Several grey colonies appeared during the second week. At the end of a month the growth was moderately good and exhibited fairly large white colonies. In a 31 days' film the bacilli varied in length from 1·5 to 3·5μ. Curved forms were not frequent. About half the bacilli were either regularly beaded or contained globular swellings.

SUMMARY.

Growth similar to that of B IX.

VIRUS—B XI.

(Bovine Mediastinal Glands.)

Strain.—RABBIT 66 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 36 days old, from Rabbit 66.

The first subcultures, made on horse and bovine serum, grew fairly well and without any initial delay.

On Horse Serum.—Growth satisfactory. In a 32 days' film the bacilli measured from ·5 to 1·25μ and averaged about ·75μ; they were straight and uniformly stained; there were some notably short and slender forms.

On Bovine Serum.—Growth as on horse serum. In a 32 days' film the length of the bacilli was from ·75 to 1·5 and occasionally 2·5μ. Some of the longer forms were curved. There were a few slender, faintly stained forms.

On Dog Serum.—A dull grey, uniform growth was produced in a week, and became copious in a fortnight. At the end of the third week whitish grey, elevated colonies appeared; these increased considerably during the fourth week. In a 15 days' film the bacilli were straight, uniformly stained, and nearly all about the same length, which was from ·75 to 1·25μ and averaged rather under 1μ.

On Glycerinated Bovine Serum.—The entire surface was covered at the end of a week with a growth which was becoming white and raised in patches; on a control tube of the same serum without the addition of glycerin the surface was uniformly, but rather more thinly covered. At the end of a fortnight there was an abundant, dull, greyish yellow layer amongst which raised colonies were appearing; the growth on pure serum was distinctly poorer. At the end of three weeks there was a further increase and the growth was dull, thick, and of the ground-glass type; the growth on pure serum was poorer. There was further increase at the end of a month, but not afterwards; the growth on pure serum remained much poorer. The ultimate yield was moist, raised, yellowish, and notably dense, quite as good as B X. In a 35 days' film there was a moderate sprinkling of straight, uniformly stained bacilli measuring from ·75 to 1μ. The rest were from 2·5 to 4·5μ long and generally curved and beaded; the beading was often quite regular.

On Broth.—A pellicle began to form at the end of the second week. The surface was covered at the end of five weeks. The pellicle was not uniform in character. There were several large, thin, opaque, uniform patches which readily became moist at the edges and soon afterwards sank. The rest of the pellicle was almost transparent, with a few opaque white spots. In a 22 days' film the bacilli exhibited marked pleomorphism. They varied in length from ·75 to 4μ. About half were curved. Very few were stained uniformly. Regularly beaded forms occurred, but were not frequent. Globular

swellings were very common and often occurred in bacilli which were otherwise faintly stained. Some of the bacilli were thicker than usual and had a clubbed extremity.

On Glycerin-Agar.—Grew fairly well, producing a moderately thick growth of the frosted-glass type at the end of three weeks, and making slow but steady progress subsequently. No wrinkling was produced. In a 24 days' film the bacilli were from 2 to 5μ in length; the great majority were curved, and more than half were irregularly stained; these either showed regular beading or contained a globular thickening which was usually centrally placed.

On Potato.—Grew fairly well. A marked increase was observed during the second week and continued during the third and fourth weeks, the final yield being fairly abundant, white, and heaped up. In a 38 days' film there were observed two types of bacilli. About two-thirds were short and straight, varying in length from 1 to 2·5μ. The remainder measured from 3 to 6μ; they were slender, generally curved, and either definitely or indefinitely beaded.

SUMMARY.

Growth similar to that of B IX.

VIRUS—B XII.

(Bovine Mediastinal Gland.)

(1.)

Strain.—G.P. 1159 from ORIGINAL MATERIAL.

Material Received.—Primary culture, 26 days old, obtained from G.P. 1,159.

The first subculture, made on horse serum, grew without any unusual difficulty.

On Horse Serum.—Growth fairly satisfactory, but never very abundant. In a 29 days' film the bacilli measured from ·5 to 1·25μ and averaged about ·75μ. They were straight and uniformly stained.

On Bovine Serum.—Growth rather poorer than the average. In a 21 days' film the bacilli resembled those grown on horse serum.

On Dog Serum.—In seven days there was only a very slight growth. In 14 days there were some isolated greyish white colonies, but the growth on the whole was very poor and patchy. In 21 days numerous white colonies had appeared. At the end of a month there was some further increase, but the growth was still rather thin and irregular. No further increase took place. In a 15 days' film the bacilli measured from ·75 to 1·5μ, occasionally 2μ, and averaged about 1μ. They were straight and uniformly stained.

On Glycerinated Bovine Serum.—Very little growth was obtained at the end of the first week; on the same serum without the addition of glycerin the growth, though not good, was better. At the end of a fortnight there was no visible change; on pure serum there was a scanty but definite growth. During the third and fourth weeks a few grey colonies made their appearance; on pure serum the growth was better, but there was only a thin layer. No increase occurred after the fourth week. The total yield was very poor, consisting of a few, isolated, grey colonies. In a 21 days' film the bacilli measured from ·75 to 2·5μ, rarely 3μ. A few were curved; very occasionally a bacillus was regularly beaded; the rest were straight and uniformly stained.

On Broth.—Growth took place with some difficulty. At the beginning of the fourth week minute specks were observed floating, and at the end of the fifth week there was a delicate speckled island about an inch in diameter. The greater part of the surface was not covered in eight weeks. In a 42 days' film the bacilli measured from 1 to 2·5μ; they were nearly all straight and uniformly stained; an occasional bacillus was slightly beaded.

On Glycerin-Agar.—Growth extremely poor, nothing but a faint grey haze being visible at the end of two months. In a 51 days' film the bacilli measured from ·75 to 2μ, the average being not over 1μ. They were straight and uniformly stained.

On Potato.—In three tubes inoculated no definite increase was observed at the end of two months.

SUMMARY.

Growth particularly poor.

(2.)

Strain.—RABBIT 98 from ORIGINAL MATERIAL.

Material Received.—Eleventh generation, 9 days old, of culture isolated from Rabbit 98.

On Bovine Serum.—Growth good. In a 21 days' film the bacilli varied in length from ·5 or ·75 to 1·5μ. They were straight and, with the exception of a few globular swellings, uniformly stained.

On Broth.—Growth commenced at the end of the third week and continued slowly, two-thirds of the surface being covered at the end of eight weeks. The growth consisted of small islands speckled with warty points which were rather closely set together. In a 34 days' film the bacilli varied in length from ·75 or 1 to 2 or 2·5μ and were straight and uniformly stained.

On Glycerin-Agar.—At the end of a month there was a thin, greyish layer with some denser points. From these points larger colonies slowly developed and extended, until the end of the tenth week. In a 27 days' film the bacilli varied in length from 1 to 3·5μ. About one quarter of the bacilli were curved, and a moderately large number either stained irregularly or showed regular beading.

On Potato.—A scanty, non-pigmented increase was observed during the first three weeks and then growth ceased. In a fifty days' film the bacilli resembled those grown on serum, but a few rather longer forms and a few beaded forms were also noted.

SUMMARY.

Growth much better than with the strain through G.P. 1159.

VIRUS—B XIII.

(Bovine Mediastinal Glands.)

Strain.—Rabbit 116 from Original Material.

Material Received.—Sixth generation, 11 days old, of culture isolated from Rabbit 116.

On Dog and Bovine Serum.—The first culture, made on dog serum, was fairly good. Subsequent cultures, made on bovine serum, grew vigorously. On bovine serum, in a 14 days' film the bacilli varied in length from ·75 to 1·5μ, occasionally 2μ, the average being at least 1μ. They were all straight and uniformly stained. Forms with a slight central constriction were frequent.

On Broth.—Growth commenced on the broth during the third week and then spread rapidly, more than a third being covered at the end of the fourth week, and nearly the whole at the end of the fifth week. The pellicle was grey, somewhat opaque, not very thin, and speckled. Shortly after the fifth week it almost all sank to the bottom. In a 31 days' film the bacilli varied in length from 1·5 to 2μ. They were all straight and uniformly stained.

On Glycerin-Agar.—Very poor growth, only a fine grey haze being obtained at the end of eight weeks. In a 41 days' film the bacilli were almost identical in appearance with those grown on bovine serum, but an occasional beaded form was observed.

On Potato.—Fine grey points were observed at the end of the sixth week, and a very slight increase took place subsequently. In a 62 days' film there were several groups of bacilli, measuring from 3 to 4μ, which were curved and uniformly stained. The rest were of the same length as those grown on serum, but some were thicker, some stained imperfectly, and some exhibited globular swellings.

Summary.

Growth very poor.

VIRUS—B XIV.

(Bovine Pleural Growth.)

Strain.—Original Material.

Material Received.—Eighth generation, 11 days old, of culture isolated from original material.

On Dog and Bovine Serum. Growth good. On bovine serum, in a 14 days' film, the bacilli measured from ·75 to 1·5μ, and were straight and uniformly stained.

On Broth.—A grey pellicle began to form early in the second week, and more than half the surface was covered at the end of the third week. The pellicle, which was thin, grey, and opaque, exhibited a marked tendency to sink and never succeeded in covering the whole surface. In a 31 days' film the bacilli varied in length from 1 to 3μ. Most of them were straight and uniformly stained; but some were curved, a few were irregularly or faintly stained. and an occasional bacillus was regularly beaded.

On Glycerin-Agar.—At the end of a fortnight there was a distinct increase, consisting of many grey streaks and warty points. Growth continued, but very slowly, and at the end of eight weeks, though the warty points were much larger, the general surface was only irregularly and scantily covered. The thickened and raised portions of the growth continued slowly to increase and extend until the tenth week. In a 42 days' film the bacilli measured from 1 to 3μ. They were nearly all straight, and very rarely beaded.

On Potato.—During the first five weeks there was some slight indication of growth, but nothing very definite. In the sixth and seventh weeks a scanty, grey growth became definitely recognisable. No change took place subsequently. In a 62 days' film the bacilli were not much longer than those grown on serum. Some were thick, and many stained faintly or irregularly.

Summary.

Growth comparable to that of Viruses B VII. and B VIII.

VIRUS—B XV.

(Bovine Mediastinal Gland.)

Strain.—G.P. 1348 from Original Material.

Material Received.—Primary culture, 32 days old, isolated from G.P. 1348.

On Dog and Bovine Serum.—The first sub-culture, made on dog serum, grew slowly and gave a very poor yield. A sub-culture from this, also on dog serum, only gave a poor yield, even at the end of a month. Subsequent cultures on dog serum commenced growth quickly and grew well. A culture on bovine serum, fourth generation, grew poorly; subsequent cultures grew well. On dog serum, in a 27 days' film, the bacilli measured from ·75 to 1·5μ, and were straight and uniformly stained. On bovine serum, in a 20 days' film, the bacilli were identical with those grown on dog serum.

On Broth.—Minute islands were observed floating at the end of the third week. These steadily extended, and the surface was nearly covered at the end of six weeks. The pellicle was very thin and semi-translucent. In a 41 days' film the bacilli varied in length from 1 to 2μ; they were straight and uniformly stained.

On Glycerin-Agar.—No more than a thin grey haze was observed until the fifth week. A slight increase then took place and continued until the ninth week, but the total yield was very scanty. In a 55 days' film, the bacilli appeared very much the same as those grown on serum, but some rather longer forms and some forms with globular swellings were noted.

On Potato.—Little, if any, growth was obtained in eight weeks. In a 35 days' film the bacilli resembled those grown on serum; very few degenerate or imperfectly stained forms were noted.

Summary.

Growth poor. Comparable to the growth of the majority of the B IV. strains.

VIRUS—B XVI.

(Bovine Mediastinal Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 71 days old, isolated from original material.

On Dog and Bovine Serum.—In the first culture made, on dog serum, there was a definite growth in 14 days, which increased to a moderate extent afterwards. The next subculture, also on dog serum, grew rather better. A culture of the third generation on bovine serum grew rather poorly. Subsequent cultures on serum grew well. On dog serum, in a 14 days' film, the bacilli measured from ·75 to 1·5μ, and averaged rather under 1μ. They were straight and uniformly stained. On bovine serum, in a 20 days' film, the bacilli varied in length from ·5 to 2μ. They were straight; occasionally a form not acid-fast was noted; otherwise the staining was uniform.

On Broth.—No growth was observed until the end of the third week. At the end of a month the surface was a quarter covered; the pellicle was thin, but slightly scaly in parts. At the end of six weeks the surface was two-thirds covered and the pellicle was beginning to sink. In a 41 days' film the bacilli varied in length from 1·5 to 3·5 or 4μ. Not more than about a sixth of them were curved. About half were beaded.

On Glycerin-Agar.—A slight but continuous growth was observed during the first three weeks. No further change was found until the end of the fifth week. During the sixth, seventh, and eighth weeks a slight further increase was visible. The ultimate yield was a delicate, finely granular growth sprinkled with some larger and slightly elevated colonies. In a 55 days' film the bacilli measured from 1 to 3·5μ. Most of them were straight. Many contained globular swellings; some were faintly stained; and some were regularly beaded.

On Potato.—A very thin, grey growth was observed at the end of the fifth week. In a 35 days' film the bacilli were nearly all straight and uniformly stained. The greater number were from 1·5 to 2μ in length, but a considerable number of groups occurred in which the bacilli were longer, from 2·5 to 3·5μ.

SUMMARY.

Growth comparable to that of Virus B XIV.

VIRUS—B XVII.

(Bovine Mediastinal Gland).

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 50 days old, isolated from original material.

On Dog and Bovine Serum.—The first subculture, made on dog serum, showed a well marked increase in six days and the total yield was fairly good. The growth on bovine serum was also satisfactory from the commencement. On dog serum, in a 14 days' film, the bacilli varied in length from ·75 to 2μ, and averaged rather over 1μ. They were straight and uniformly stained. On bovine serum, in a 20 days' film, the bacilli were, on the whole, slightly shorter.

On Broth.—A few small, delicate islands appeared towards the end of the third week. A thin, speckled film covered two-thirds of the surface at the end of the fifth week and then began to sink. In a 41 days' film the bacilli varied in length from 1 to 3μ. The greater number of them stained irregularly; many were beaded and many contained globular swellings. A small proportion were curved.

On Glycerin-Agar.—No more than a thin, grey haze was observed until the sixth week. The growth then became very slightly denser. In a 55 days' film the bacilli varied in length from ·75 to 3μ, the majority being less than 2μ. Only a small number were curved. There were some beaded and some imperfectly stained forms, but the great majority were stained uniformly.

On Potato.—A very scanty growth was noted in the sixth week. In a 41 days' film the bacilli were very similar to those grown on serum.

SUMMARY.

Growth poor; comparable to B IV. (Original Material).

VIRUS—B XVIII.

(Bovine Bronchial Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, on egg, 39 days old, isolated from original material.

On Dog and Bovine Serum.—The first culture, made on dog serum, was slow in starting, but produced a moderate amount of growth at the end of four weeks. This was subcultured on to bovine serum, where it grew fairly well, but slowly. Later generations, on both dog and bovine serum grew readily. On dog serum, in a 27 days' film, the bacilli varied in length from ·75 to 1·5μ (occasionally 2μ). They were straight and uniformly stained. On bovine serum, in a 20 days' film, the bacilli were identical with those grown on dog serum.

On Broth.—Growth commenced at the end of the third week, and covered the surface at the end of the sixth week. The pellicle was very thin, semi-translucent, and speckled with grey points. In a 41 days' film the bacilli were very similar to those grown on serum, but some rather longer and thicker forms were noted.

On Glycerin-Agar.—A delicate grey haze was formed during the second and third weeks; this grew more opaque during the seventh and eighth weeks, and became slightly thickened in patches. In a 55 days' film the bacilli varied in length from ·75 to 3·5μ. Many of them stained very irregularly, and some were beaded. The great majority were straight.

On Potato.—There was no definitely visible growth at the end of five weeks. In a 35 days' film the bacilli resembled, on the whole, those grown on serum; but longer forms and some curved and beaded forms were also present.

SUMMARY.

Growth poor; comparable to B XVII.

VIRUS—B XIX.

(Bovine Bronchial Gland.)

Strain.—Original Material.

Material Received.—Fourth generation, 8 days old of culture isolated from original material.

On Bovine Serum.—The tube received only contained a scanty amount of growth. The subculture from this covered the surface with a thin layer in a week and gave a good yield at the end of three weeks. In a 16 days' film the bacilli varied in length from ·75μ, or rather less, to 1·5μ; many slender forms were noted; very few bacilli were curved.

On Broth.—In one flask no growth appeared on the surface until the end of the sixth week; rapid increase then took place, and the surface was completely covered before the end of the eighth week. In another flask floating islands were formed at the end of the third week and the greater part of the surface was covered at the end of the fifth week. The pellicle was very delicate, nearly translucent on the whole, and speckled with some minute grey nodules. In a 46 days' film the bacilli measured from 1 to 2·5 or 3μ; they were all uniformly stained and only a few were curved.

On Glycerin-Agar.—Only a slight grey haze was present at the end of eight weeks. In a 47 days' film the bacilli varied in length from ·75 to 2μ; they were nearly all straight; many of them were beaded.

On Potato.—A very slight growth was observed during the first fortnight; no increase took place subsequently. In a 46 days' film the bacilli varied in length from 1 to 3μ; most of them were straight; beading was rather frequent.

Summary.

Growth particularly poor on broth and glycerin-agar.

VIRUS—B XX.

(Bovine Mediastinal Gland.

Strain.—G.P. 1471 from Original Material.

Material Received.—Primary culture, on egg, 13 days old, isolated from G.P. 1471.

On Bovine Serum.—Grew well from the commencement. In an 18 days' film the great majority of the bacilli varied in length from ·75 to 1·5μ, and were straight and uniformly stained. There were a few curved forms, measuring from 2 to 2·5μ.

On Broth.—Growth commenced at the end of the first week and the surface was covered at the end of the third week. The pellicle was very thin on the whole, but exhibited a fewer denser points. During the fourth and fifth weeks it became moist and began to sink. In a 15 days' film the bacilli varied in length from 1·5 to 3·5μ, with the exception of occasional forms which were much longer. The average length was about 2·5μ. Curved forms were numerous. Many bacilli showed globular swellings and some were beaded. There were some faintly stained forms and a few were not acid-fast.

On Glycerin-Agar.—A moist greyish layer, thicker in some patches than in others, but nowhere very dense, developed in the course of the first five weeks. Growth then ceased. In a 31 days' film the bacilli varied in length from 1·5 to 3·5μ. The shorter forms were straight and uniformly stained, the longer curved and beaded.

On Potato.—Slight, non-pigmented increase during the first fortnight only. In a 31 days' film the bacilli varied in length from 1·5 to 5μ, forms above 3·5μ being infrequent. The majority were curved and beaded.

Summary.

Growth comparable to that of B XVI.

VIRUS—B XXI.

(Bovine Bronchial Gland.)

Strain.—Original Material.

Material Received.—Second generation, 24 days old of culture isolated from original material.

On Dog and Bovine Serum.—On each of these media the first culture was poor. Subsequent cultures, made on bovine serum, were good. In a 21 days' film, from bovine serum, the bacilli varied in length from ·5 to 1·5 and occasionally 2μ; they were straight and uniformly stained.

On Broth.—Growth commenced at the end of the third week and the surface was covered with a delicate semi-translucent pellicle at the end of the sixth week. In a 39 days' film the bacilli were from 1 to 2μ in length; they were uniformly stained and nearly all straight.

On Glycerin-Agar.—A slight grey haze was all that was visible at the end of eight weeks. In a 47 days' film the bacilli varied in length from ·75 to 2·5 or 3μ; they were uniformly stained and with the exception of a few curved forms were straight.

On Potato.—No visible growth was obtained at the end of eight weeks. Microscopic examination, after 57 days' incubation, showed that the bacilli retained the typical appearance of bacilli grown on serum.

Summary.

Growth very poor; comparable to B XIX.

VIRUS.—B XXII.

(Bovine Portal Gland.)

(1.)

Strain.—G.P. 1509 from Original Material.

Material Received.—Primary culture, on egg, 36 days old, isolated from G.P. 1509.

On Bovine Serum.—Growth satisfactory. In a 19 days' film the bacilli varied in length from ·75 to 1·5 or 2 μ and were straight and uniformly stained.

On Broth.—Growth commenced at the beginning of the third week and the surface was covered at the end of the fifth week. The pellicle was thin, nearly transparent, and fragmentary. In a 41 days' film there were a few coccal forms, but most of the organisms varied in length from 1·5 to 3·5 and occasionally 4·5μ. Curved and beaded forms were not very common.

On Glycerin-Agar.—A grey haze interspersed with some minute isolated warts, due to increase of small lumps of material inoculated. In a 44 days' film there were some short, thick, almost oval forms measuring from ·75 to 1μ. The rest of the bacilli varied in length from 1·5 to 5μ and were very irregular. Curved, beaded, and faintly stained forms were all frequent; and both globular swellings and cylindrical, deeply stained thickenings were common.

On Potato.—A scanty, pale growth was obtained at the end of six weeks but showed no subsequent increase. In a 51 days' film the bacilli varied in length from ·75 to 4 and occasionally 5μ. Nearly all the longer forms were beaded, and frequently curved. Some bacilli were stained irregularly.

Summary.

Growth poor; slightly better than B XXI.

(2.)

Strain.—Dog 72 from Original Material.

Material Received.—Third generation, 10 days old, of culture isolated from the lung of Dog 72.

On Horse Serum.—Growth good. In an 18 days' film the bacilli varied in length from ·75 to 1·5μ and were straight and uniformly stained.

On Broth.—Growth was very slow, the surface being not more than two-thirds covered in two months. The pellicle was opaque, thin at first, and then gradually became thicker. It showed no tendency to sink. In a 38 days' film the bacilli varied in length from 1 to 3·5μ. The longer forms were frequently curved and beaded.

On Glycerin-Agar.—Growth took place quickly during the first fortnight, forming a fairly dense grey layer which had spread on to the glass. Afterwards, the rate of increase was much slower and the total yield was not very abundant. In a 42 days' film the bacilli varied in length from 1 to 5μ. Curved forms were frequent; the staining was very irregular, and darkly stained thickenings were common.

On Potato.—A grey, crumb-like, scanty growth was slowly formed. In a 56 days' film the bacilli varied in length from 1 to 5 or 6μ. The majority were curved. Staining was very irregular.

Summary.

Growth distinctly more luxuriant than the strain through G.P. 1509 from original material.

VIRUS.—B XXIII.

(Bovine Lung.)

Strain.—Original Material.

Material Received.—Primary culture, 43 days old, isolated from original material.

On Bovine Serum.—Growth good. In a 19 days' film the bacilli varied in length from ·75 to 2·5μ; a few were curved and an occasional form was beaded.

On Broth.—Growth commenced at the beginning of the third week, and at the end of the fourth week the surface was about half covered with a pellicle which was partly dense, grey, and moist, and partly thin and semi-translucent. The denser portions then began to sink, whilst the thinner portions continued to grow but failed to completely cover the surface. In a 22 days' film the bacilli varied in length from 2 to 3·5μ. More than half were curved; many had a tendency to beading.

On Glycerin-Agar.—A fine grey haze, with a few denser points. In a 44 days' film the bacilli varied in length from 1·5 to 6μ. The longer forms were all curved and either regularly beaded or with irregular thickenings.

On Potato.—No growth was visible. In a 51 days' film a few clumps of bacilli, which were obviously part of the material inoculated, were found; there was no indication of growth.

Summary.

Growth very poor; comparable to B XXI.

VIRUS—B XXIV.

(Bovine Lung, Spleen and Sternal Gland.)

(1.)

Strain.—Original Material (Lung).

Material Received.—Primary culture, 19 days old, isolated from original material.

On Bovine Serum.—Growth poor for first four generations then gradually improving. In a 19 days' film the bacilli varied in length from ·75 to 2·5μ. Most of them were straight and uniformly stained, but there were occasional curved forms and occasional forms with globular swellings.

On Broth.—The surface was covered in five weeks with a pellicle which was thin and transparent on the

whole but contained some opaque, flaky patches. In a 41 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—A fine grey haze. In a 27 days film the bacilli, with the exception of some slightly longer and irregular forms, resembled those grown on serum.

On Potato.—A very slow and scanty growth was obtained, presenting the appearance, at the end of eight weeks, of very minute, dry, nearly white crumbs. In a 15 days' film the bacilli varied in length from 1 to 3·5μ. The longer were curved. The staining was generally uniform, and there was no tendency to beading, but there were a few globular swellings.

SUMMARY.

Growth very poor; comparable to B XXIII.

(2.)

Strain.—G.P. 1548 from ORIGINAL MATERIAL (SPLEEN AND STERNAL GL.).

Material Received.—A portion of the spleen of G.P. 1548. In a smear preparation bacilli were somewhat scanty, from two to six being found in every five or six microscopic fields. They measured from 2 to 3 μ; most of them were beaded, and they were generally curved.

On Dog Serum.—Minute colonies, dotted all over the surface, were distinctly visible in nine days after inoculation with an emulsion of the spleen. These colonies increased in size and number during the second and third weeks, and then development ceased. In a tube which was left untouched after inoculation the ultimate yield consisted of a granular growth covering the entire surface and consisting of discrete colonies which were closely set together and were from $\frac{1}{4}$ to $\frac{1}{2}$ a millimetre in diameter.

On Bovine Serum.—Four tubes were inoculated with an emulsion of the spleen. In all of them, as is usual with bovine serum but not with dog serum, a scum formed over the surface before any growth appeared. In one of the tubes colonies were just visible, emerging from the scum, but not so numerous as on the dog serum tubes, in 9 days. The other three tubes showed no growth, visible to the naked eye, in 12 days; they were then rubbed up with the platinum loop, and two days afterwards a definite growth appeared all over the surface in all of them; at the end of three weeks a fairly thick layer was obtained, quite as copious as on any of the dog serum tubes. In subcultures, made on bovine serum, the bacilli grew readily. In a 13 days' film the bacilli varied in length from ·75 to 1·5 or 2μ and were straight and uniformly stained.

On Glycerinated Bovine Serum.—Three tubes were inoculated, but though the surface was rubbed up no sign of growth appeared after two months' incubation.

On Broth.—Growth commenced at the beginning of the third week and the surface was covered at the end of the fifth week. The pellicle was thin and semi-transparent on the whole, but interspersed with opaque flakes and streaks of denser material. In a 41 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—A fine grey haze, with a few isolated, denser points was obtained. In a 44 days' film the bacilli varied in length from ·75 to 3 or 3·5μ. The longer forms were infrequent and were curved and often partially beaded. The rest were straight and uniformly stained.

On Potato.—No growth was visible at the end of a month. At the end of six weeks a very scanty, pale growth was recognisable. No further increase took place. In a 51 days' film the majority of the bacilli resembled those grown on serum. There were a few longer, regularly beaded forms.

SUMMARY.

Growth somewhat better than strain (I.) of this virus.

VIRUS—B XXV.

(Bovine Pleura.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Sixth generation, 13 days old, of culture isolated from original material.

On Bovine Serum.—Growth good. In a 19 days' film the bacilli were uniformly stained and, with rare exceptions, straight. They measured from ·75 to 1·5 and occasionally 2μ in length.

On Broth.—Growth commenced at the end of the first week; at the end of the second week the surface was two-thirds covered; and at the end of the third week it was completely covered. The pellicle was opaque, not of equal density but with numerous dense patches; these patches very readily became moist and fell to the bottom of the flask. In a 22 days' film the bacilli varied in length from ·75 to 3μ. Curved forms were rather rare. More than half the bacilli were either regularly beaded or stained irregularly.

On Glycerin-Agar.—During the first three weeks growth was fairly rapid, the surface becoming covered with an opaque grey layer which showed numerous small, raised colonies. After this period the rate of progress was very slow. The ultimate yield showed several dense grey patches, but the intervening surface between these was only thinly covered. In a 42 days' film the bacilli varied in length from 1 to 3.5μ. Many bacilli were curved. Thickened extremities were common and many bacilli contained conspicuous oval bodies.

On Potato.—Small, pale colonies were formed in the third week, and at the end of the sixth week most of the surface was covered with a white, evenly distributed layer. In a 57 days' film the bacilli varied in length from 1 to 2μ. Most of them were straight. The staining of some was irregular.

SUMMARY.

Growth comparable to that of B V, but not quite so good.

VIRUS.—B XXVI.

(Bovine Bronchial Glands.)

Strain.—Original Material.

Material Received.—Fifth generation, 9 days old, of culture isolated from original material.

On Bovine Serum.—Growth good. In a 9 days' film the bacilli varied in length from $\cdot 75$ to 2 or $2 \cdot 5\mu$. A few were curved; a few were faintly or irregularly stained, and in some there was a slight tendency to beading.

On Broth.—Growth commenced at the beginning of the third week and the surface became covered in the sixth week. The pellicle consisted of thin, partially translucent material speckled with numerous grey warts. In a 27 days' film the bacilli resembled serum-grown bacilli but were rather longer, the length ranging from 1 to 3μ.

On Glycerin-Agar.—A thin grey layer slowly developed. At the end of nine weeks the growth was still thin, but opaque, and presented a fine, ground glass appearance. In a 42 days' film the bacilli varied in length from $\cdot 75$ to 3μ. They were almost all straight. Globular swellings were numerous.

On Potato.—A scanty but definite, pale yellow growth appeared during the third week, and then continued to grow very slowly. At the end of nine weeks the greater part of the surface was covered with a thin cream-coloured growth, slightly raised in patches. In a 42 days' film the bacilli varied in length from 1 to $2 \cdot 5$ or 3μ. Most of them were straight, there was some tendency to beading.

Summary.

Growth, on the whole, comparable to that of B XVI.

VIRUS.—B XXVII.

(Bovine Bronchial Gland.)

Strain.—Original Material.

Material Received.—Third generation, 14 days old, of culture isolated from original material.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli varied in length from $\cdot 5$ to 2μ, and were straight and uniformly stained.

On Broth.—An opaque, moist pellicle commenced to grow rapidly in the second week. It began to sink after the surface was about two-thirds covered. At the end of the third week nearly all of it had fallen to the bottom. In a 24 days' film the bacilli varied in length from 1 to 3μ. The majority of the bacilli were straight and uniformly stained.

On Glycerin-Agar.—A fine grey haze with some denser, raised patches, which appeared after many weeks. In a 42 days' film the bacilli varied in length from 1 to $3 \cdot 5\mu$ and were mostly straight. Many were stained unequally.

On Potato.—A scanty, white growth was obtained in six weeks. In a 49 days' film the bacilli varied in length from $\cdot 75$ to $3 \cdot 5\mu$. Most of them were straight. The longer forms had a tendency to be stained irregularly.

Summary.

Growth, on the whole, poorer than B XXVI.

VIRUS—B XXVIII.

(Butcher's Meat).

Strain.—Original Material.

Material Received.—Second generation, 10 days old, of culture isolated from original material, a lymphatic gland found in butcher's meat.

On Horse Serum.—Growth satisfactory. In an 18 days' film the bacilli varied in length from $\cdot 5$ to $1 \cdot 5\mu$ (occasionally 2 to $2 \cdot 5\mu$.) They were straight and uniformly stained, with the exception of occasional curved and beaded forms.

On Broth.—Many minute islands appeared during the second week and subsequently. These readily became moist and sank. The surface never became uniformly covered. In a 40 days' film the bacilli varied in length from 1 to $3 \cdot 5\mu$. Most of them were straight. Darkly stained thickenings were extremely common.

On Glycerin-Agar.—A scanty, greyish growth appeared in a fortnight. A slight increase took place subsequently. At the end of two months the growth was still scanty on the whole, but a few denser, granular patches were present. In a 41 days' film long, slender, curved forms (5 to 6μ or even longer) were very numerous. The rest of the bacilli varied in length from 1 to 4μ. Most of the bacilli were curved and irregularly stained.

On Potato.—A moderate amount of grey growth was slowly formed. In a 56 days' film the bacilli varied in length from $\cdot 75$ to 5 or 6μ. They were mostly curved and stained irregularly, though many were regularly beaded.

Summary.

Growth very poor on broth; rather scanty on glycerin-agar and potato.

VIRUS—B XXIX.

(Bovine Mesenteric Gland)

Strain.—ORIGINAL MATERIAL.

Material Received.—Sixth generation, 5 days old, of culture isolated from original bovine material.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ, averaging slightly over 1μ. They were straight and uniformly stained.

On Broth.—The surface was covered in three weeks with a thin, grey pellicle which soon became moist and sank. In a 21 days' film the bacilli varied in length from 1 to 3·5μ. Staining was very irregular. Straight forms were more numerous than curved.

On Glycerin-Agar.—Growth was rapid during the first fortnight and a fairly dense but unequally distributed layer was produced. After the third week there was no further increase. In a 45 days' film the bacilli varied in length from 1 to 3μ. They were straight and often irregularly stained.

On Potato.—A moderate amount of growth was obtained. It was grey during the first month, but afterwards became yellow. In a 56 days' film the bacilli measured from 1 to 4μ. The majority were straight. Irregular forms were not very numerous.

SUMMARY.

On broth a pellicle was readily formed but soon afterwards sank; on glycerin-agar growth commenced well, but was not maintained after the third week; on potato growth was fairly good.

VIRUS—B XXX.

(Bovine Inguinal Gland)

Strain.—ORIGINAL MATERIAL.

Material Received.—Primary culture, 35 days old, obtained from the original bovine material.

On Horse Serum.—Growth rather poor. In a 14 days' film the bacilli varied in length from ·75 to 2·5μ. A few were curved.

On Broth.—Growth was slow during the first fortnight, but during the third week a thin, grey, uniform pellicle spread over the whole surface. Soon afterwards it became moist and sank. In a 21 days' film the bacilli varied in length from 1 to 3μ. The longer were slightly curved; a few were beaded, but the staining on the whole was regular.

On Glycerin-Agar.—No more than a grey haze was formed. In a 47 days' film the bacilli resembled those grown on serum.

On Potato.—A very scanty, hardly perceptible layer of growth was formed. In a 56 days' film the bacilli varied in length from 1 to 4μ. Curved and beaded or irregularly stained forms were fairly common (about 20 per cent.).

SUMMARY.

Growth very poor on glycerin-agar, but rather better on broth.

VIRUS—H 2. Sp. A.

(Mixed Human Sputum.)

(1.)

Strain.—G.P. 534 from CALF 93.

Material Received.—Twelfth generation, 6 days old, of culture isolated from G.P. 534, which was inoculated from Calf 93.

On Bovine Serum.—Growth luxuriant. In a 24 days' film the bacilli varied in length from ·75 or rather less to 1·5μ or rather more. They were straight and uniformly stained.

On Broth.—At the end of a fortnight the surface was covered with an opaque, rather thin, grey pellicle which was somewhat warty in parts. The pellicle did not increase much in thickness, and at the end of a month showed a tendency to curl up at the edges and sink. In a 29 days' film the bacilli varied in length from 1 to 2·5μ. The greater number were short, rather thick, and uniformly stained. There were also some irregularly stained and some curved forms.

On Glycerin-Agar.—Growth took place rapidly during the first fortnight and then continued slowly until about the end of the fifth week. It was grey, moist, and of moderate density. In a 19 days' film the bacilli varied in length from 1·5 to 3μ. Many were curved; regular beading and globular swellings were frequent; some forms were not acid-fast.

On Potato.—Growth occurred during the first month. It was rather scanty and of a pale, cream-yellow colour. In a 53 days' film the bacilli varied in length from ·5 to 3μ. Some of the smallest forms looked almost like cocci. Most of the bacilli were straight; some of them were regularly beaded; globular swellings were numerous.

SUMMARY.

Growth good on serum; on broth rapid, only moderately dense, and with a tendency to become moist and sink; on glycerin-agar and potato moderately good.

(2.)

Strain.—G.P. 690 from CALF 111.

Material Received.—Fourth generation, 34 days old, of culture isolated from G.P. 690, which was inoculated from Calf 111.

On Horse Serum.—The first and second cultures made were poor, probably owing to the material received not being in a particularly vigorous condition. Subsequent cultures grew readily and abundantly, forming a thick layer all over the surface, with many raised colonies. In a 14 days' film a few of the bacilli were curved and a still smaller number were beaded. The rest were straight

and uniformly stained. The majority of the bacilli varied in length from 2 to $\cdot 75\mu$ but some were rather shorter than $\cdot 75\mu$ and a few were unusually long (4μ to 3μ.) The average length was slightly under $1\cdot 5\mu$. In a 28 days' film of the same culture no appreciable difference in morphology was noted. In an 18 days' film from another culture the same appearances were noted, the bacilli being again rather longer and rather more frequently curved and beaded than is usual in serum cultures.

On Bovine Serum.—The first and second cultures were poor. Subsequent cultures grew very abundantly with the formation on good, adult, bovine serum, of highly pigmented yellow colonies. The morphology of the bacilli, in a 9 days' film, was the same as on horse serum, with the exception that no beaded forms were noted.

On Broth.—In 14 days the surface was well covered with a thin but grey and opaque pellicle, speckled with white warts and small crusts. Subsequently the pellicle increased somewhat in general density, spread on to the walls of the flask, and became more prominently warty. From observations of other flasks inoculated with this strain it was found that the characters of the growth on broth were not constant. In some flasks the pellicle grew very rapidly, was slightly opaque, but almost uniformly thin, and did not subsequently thicken; in other flasks growth was slower but the pellicle much denser, and the surface ultimately became covered with a uniformly thick, warty layer. In a 14 days' film the bacilli stained uniformly, with the exception of some which showed globular swellings. Straight and curved forms were about equally numerous. The length varied from $1\cdot 5$ to 3μ, the average being a little over 2μ. In a 29 days' film from the same culture no morphological differences from the 14 days' film were noted.

On Glycerin-Agar.—Growth progressed steadily but rather slowly. An opaque grey layer was formed in the second week; this gradually became denser and showed on the surface a granular appearance, due to the presence of numerous minute raised colonies and crusts. About the sixth week some larger, prominently projecting colonies appeared, and the growth began to spread on to the glass of the tube. The growth, on the whole, was good, but was neither so rapid, dense, warty, or wrinkled as that obtained with many more luxuriantly growing viruses. In a 19 days' film the bacilli were in the majority of cases uniformly stained, though both regularly beaded forms and forms with globular swellings were found. Curved forms were more frequent than straight. The bacilli varied in length from $1\cdot 5$ to 5μ.

On Potato.—A crop of yellow colonies appeared during the second week and increased during this and the following week. Very little increase took place subsequently. The colonies did not become very large or heaped up, and the surface intervening between them was only thinly covered. In a 34 days' film the majority of the bacilli were straight, uniformly stained, and measured from $\cdot 5$ to $1\cdot 5\mu$. Forms from 2 to 3μ, curved and generally beaded, were present in very small numbers. In a 31 days' film from another culture no morphological differences were noted.

SUMMARY.

Growth rather better than that of the strain from Calf 93.

VIRUS—H 7. C.M.

(Human Mesenteric Glands.)

(1.)

Strain.—G.P. 552 from Cow 73.

Material Received.—Seventh generation, 31 days' old, of culture isolated from G.P. 552, which was inoculated from Cow 73.

On Horse Serum.—At first a little difficulty was experienced, owing to the slowness of the growth in starting. and the formation of a scum on the medium before the bacilli had covered the surface. Later cultures gave no trouble; they readily yielded a moderately dense layer of growth thickly set with small, grey, elevated colonies. In a 14 days' film the bacilli, with few exceptions, were straight and uniformly stained. They varied in length from about 2μ to rather less than $\cdot 75\mu$, the average being about 1μ. In a 28 days' film of the same culture no change was observed.

On Bovine Serum.—As on horse serum, the first one or two cultures were poor, later cultures were good. In a 14 days' film the bacilli were on the whole similar, morphologically, to those grown on horse serum, but, perhaps, a little longer.

On Dog Serum.—A moist, grey growth, without any raised colonies, was produced in a week. At the end of the second week the growth was fairly copious, and some discrete, white colonies were beginning to form. These increased in size and number, became more raised, and coalesced into patches during the third and fourth weeks. In a 15 days' film the bacilli were all uniformly stained and nearly all straight; they measured from $\cdot 75$ to $1\cdot 5\mu$, the average being rather less than 1μ.

On Glycerinated Bovine Serum.—At the end of a week there was a dull, fairly thick growth over the entire surface; the growth on the same bovine serum to which no glycerin had been added was identical in character and amount. At the end of a fortnight the growth had increased, and was definitely greyish and slightly heaped up; on pure bovine serum the growth was very much the same in type, but rather less abundant. At the end of three weeks there was a further increase, and the growth had assumed a frosted glass appearance; on pure bovine serum the growth was only moderately dense, and was dull and uniform. At the end of a month the growth had become whiter and more heaped-up; on pure bovine serum the growth was drier, more uniform, and less copious. No further change took place. In a 20 days' film the bacilli measured from 1 to $2\cdot 5\mu$; a moderate number were curved; there were a few globular swellings and a few regularly beaded forms.

On Broth.—The broth was covered in about a fortnight, and the growth then spread on to the glass. The pellicle was delicate, grey, for the most part opaque, and speckled all over with small, slightly raised, white points. After this stage was reached, a good deal of deposit began to be formed, and the pellicle showed no tendency to become uniformly thicker, or warty, or crusty. In a smear from a 43 days' culture the bacilli were homogeneously and deeply stained; most of them were straight; their length was from $1\cdot 5$ to $2\cdot 5\mu$, the great majority not being longer than $1\cdot 5\mu$. In a film from a 25 days' culture the same general characters were observed, but the bacilli were rather longer, and more of them were curved.

On Glycerin-Agar.—In 7 days very fine, greyish, semi-translucent points appeared all over the surface. In 14 days half the surface was distinctly opaque, and the whole was speckled with minute grey colonies. In 21 days the growth had increased in thickness, and had a grey, frosted appearance at the margins; there were no large colonies. In 28 days the growth showed no large colonies, was not wrinkled, but had a general appearance of frosted glass; it was beginning to spread on to the sides of the test-tube. Subsequent to this period the characters of the cultures remained unchanged. In a film from a 42 days' growth nearly all the bacilli were short, straight, or only slightly curved, and uniformly stained. The length ranged from $1\cdot 5$ to $2\cdot 5\mu$, the average being rather less than 2μ.

On Potato.—On a good potato some minute grey colonies were found in a week's growth; during the second and third weeks the increase was very slight; about the fourth week the increase began to be more marked, but as it was pale and tended to be uniformly distributed, it was better realised by removing a sample with the platinum loop than by mere inspection of a tube. The final yield was somewhat scanty and non-pigmented. In films from two cultures, one 42 and the

other 33 days old, the characters of the bacilli proved to be the same. Rather more than half measured from ·75 to 2μ, and were generally straight and uniformly stained. Of the rest, measuring from 2·5 to 4·5μ, the majority were curved and unequally stained, exhibiting either regular beading or globular swellings.

Summary.

Growth on serum and glycerinated serum fairly good; on broth rapid but thin, and with a tendency to sink; on glycerin-agar and potato rather poor.

(2.)

Strain.—G.P. 646 from Calf 103.

Material Received.—Sixth generation, 69 days old, of culture isolated from G.P. 646, which was inoculated from Calf 103.

On Horse Serum.—Growth satisfactory. In a 15 days' film the bacilli stained uniformly, were nearly all straight, and measured on the average rather less than 1μ.

On Bovine Serum.—Growth satisfactory. Morphology in a 22 days' film, as on horse serum; but a few rather longer forms were noted.

On Broth.—Growth took place rather slowly. About the middle of the third week small thick patches began to stream off the serum; these gradually increased and covered the greater part of the surface in about six weeks, forming large discrete islands which sank on the slightest disturbance. In a 46 days' film the bacilli measured from 1 to 3μ, were for the most part straight, and stained very irregularly, globular swellings, thickened extremities, and faintly stained forms all being numerous.

On Glycerin-Agar.—A slow, steady growth took place, forming a moderately thick, not wrinkled layer of the frosted glass type in five weeks, and becoming gradually denser during the sixth and seventh weeks. In a 32 days' film the bacilli exhibited marked pleomorphism. They measured from 1 to 4·5μ; about one-third were curved; nearly all showed globular swellings, many were faintly stained, and a few were regularly beaded.

On Potato.—A poor, but definite growth was obtained. It was first noticed at the end of a fortnight, and increased slightly until the end of the fifth week. It was thin, grey, and spread over the greater part of the surface. In a 39 days' film the bacilli measured from 1·5 to 3·5 or 4μ. Most of them were curved, irregularly stained, and showed a tendency to beading.

Summary

Growth similar, on the whole, to the strain through Cow 73.

VIRUS—H 8. S.C.

(Human Mesenteric Glands.)

(1.)

Strain.—Original Material.

Material Received.—Twelfth generation, 15 days old, of culture isolated from original material

On Horse Serum.—Growth satisfactory. In a 24 days' film the bacilli measured from 1 to 2·5μ, the average being about 1·5μ; the bacilli were rather slender; some of the longer forms were curved; none were regularly beaded; some exhibited globular swellings.

On Bovine Serum.—Growth satisfactory. In a 28 days' film the bacilli resembled, on the whole, those grown on horse serum, the only difference being that forms as short as ·75μ were also fairly frequent and globular swellings were rare.

On Broth.—Towards the end of the second week a somewhat dense pellicle began to stream off the entire margin of the serum. The broth was completely covered with a fairly tough but unequally dense pellicle at the end of a month. In a 15 days' film the bacilli measured from 1·5 to 2.5μ. Almost all were uniformly stained; only a few were curved.

On Glycerin-Agar.—An even, grey layer of growth was formed in a fortnight. This became definitely wrinkled at the end of the third week, and at the end of the fifth week the wrinkles formed a prominently raised network. In a 34 days' film the bacilli measured from 1 to 2·5μ. The shorter forms were uniformly stained, straight, and often with a central constriction. The longer forms were often beaded, or showed globular swellings, and were often curved.

On Potato.—A fairly copious, yellow growth was produced in a fortnight. This increased vigorously, and became heaped up during the third and fourth weeks. In a 30 days' film the bacilli measured from 1·5 to 3·5μ, with occasional longer forms. About half the bacilli were curved. Nearly every bacillus was either regularly beaded or exhibited a globular swelling, each type being almost equally frequent.

Summary.

Growth on broth dense but unequal, with no tendency to sink; growth on glycerin-agar thick and wrinkled; growth on potato pigmented and abundant.

(2.)

Strain.—Calf 361.

Material Received.—Primary culture, 46 days old, isolated from Calf 361.

On Horse Serum.—Growth good. In a 20 days' film the bacilli were all uniformly stained and with few exceptions straight. Their length varied from 2 to ·5μ, the average being 1μ.

On Bovine Serum.—Growth as on horse serum. In a 20 days' film the bacilli were slightly longer than on horse serum.

On Broth.—Growth commenced at the end of the first week and the surface was covered at the end of the fifth week. The pellicle was dense but not uniformly dense. In a film from a 38 days' growth almost all the bacilli were deeply and homogeneously stained; there were no beaded forms. The length varied from 1 to 3μ, and was generally between 2 and 3μ. Curved and slightly curved forms were frequent, but straight bacilli were much more numerous.

On Glycerin-Agar.—For the first fortnight the growth was vigorous. It subsequently advanced with less rapidity, and yielded at the end of a month a fairly copious growth of the frosted glass type, interspersed

with a moderate number of rather small projecting colonies. The growth gradually increased in density during the two subsequent weeks. In a 38 days' film the bacilli were, on the whole, uniform; their length was from 1 to 3μ, generally between 2 and 3μ; curved and slightly curved forms were frequent, but straight bacilli were much more numerous.

On Potato.—A fairly dense crop of grey colonies appeared at the beginning of the second week and gradually began to acquire a yellow tinge. The ultimate yield was a not very copious, pale yellow, warty growth. In a 31 days' film the bacilli for the most part were long (3 to 7μ), curved, and uniformly stained or with only a slight tendency to beading.

SUMMARY.

Growth, on the whole not quite so good as with the strain from the original material.

VIRUS—H 9. C.T.

(Human Wrist Joint.)

Strain.—G.P. 996 from CALF 183.

Material Received.—The first culture received was 136 days old. This proved to be dead. The second culture received was the fifth generation, 20 days old, of a culture isolated from G.P. 996, one of the fourth series of guinea-pigs inoculated from Calf 183.

On Horse Serum.—A rapid and very abundant growth was obtained without any difficulty. In a 20 days' film the bacilli were all uniformly stained and almost all straight. Their length varied from 2 to $\cdot 5\mu$, the average being less than 1μ.

On Bovine Serum.—Growth very abundant. In a 20 days' film the only difference noted from the bacilli grown on horse serum was that forms as long as 2μ were rather more frequent.

On Dog Serum.—Abundant growth, commencing to be raised and white in a week's time. In a 14 days' film the bacilli resembled those grown on bovine serum.

On Glycerinated Bovine Serum.—At the end of a week there were numerous raised, yellow colonies, but the rest of the surface was rather thinly covered. The entire surface was well covered at the end of a fortnight, and the growth had become fairly thick, yellow, and raised at the end of a month. No further increase took place after this period. In a 20 days' film the bacilli measured from 1 to 3μ, and were all uniformly stained; about one-third of the longer forms were curved.

On Broth.—Growth took place very quickly, a delicate film being found on the greater part of the surface in from three to four days. The pellicle became denser as the growth continued. First, opaque white spots appeared, and then gradually the growth became more or less uniformly opaque and tough. It exhibited no tendency to sink. In a 23 days' film the bacilli were uniformly stained and more frequently straight than curved. Their length varied from 1 to 3μ, and averaged about $1\cdot 5\mu$.

On Glycerin-Agar.—A grey opaque layer covered the surface in the second week. This continued to grow rapidly until about the end of the fifth week; as it grew, it first became very wrinkled all over the surface and later on developed some projecting colonies of moderate size. In a 23 days' film the bacilli were of various characters. Forms of 1μ or less were very frequent; curved, not beaded, forms about 2μ long, were numerous; curved forms (2 to 3μ, with globular swellings were moderately numerous; and regularly beaded, curved forms from 3 to 4μ long were not uncommon.

On Potato.—Slow increase took place during the first four weeks with the formation of a moderately dense, pale, lemon-yellow layer. No subsequent increase took place. In a 31 days' film, with the exception of a few straight forms about 1μ long, the bacilli were mostly curved, from $2\cdot 5$ to 5μ in length, and sometimes regularly beaded; very frequently they contained globular swellings.

SUMMARY.

Growth good on serum and glycerinated serum; good, but somewhat irregular, on broth; good on glycerin-agar; only moderately good on potato.

VIRUS—H 10. B.S.

(Human Mesenteric Glands.)

(1.)

Strain.—G.P. 771 from ORIGINAL MATERIAL.

Material Received.—Eighth generation, 15 days old of culture isolated from G.P. 771, which was one of the fourth series of guinea-pigs inoculated with the original material.

On Horse Serum.—Growth satisfactory. In a 14 days' film the bacilli measured from $\cdot 5$ to $1\cdot 25\mu$ in length, the average not exceeding $\cdot 75\mu$; they were all straight and uniformly stained.

On Bovine Serum.—Growth satisfactory. Morphology of bacilli, in a 28 days' film, as on horse serum.

On Dog Serum.—An abundant, dull grey growth covered the surface at the end of a week. The growth continued to increase until the fourth week and then exhibited many raised white patches. In a 15 days' film the bacilli measured from $\cdot 75$ to $1\cdot 5\mu$, averaging about 1μ; they were straight and uniformly stained.

On Glycerinated Bovine Serum.—Throughout the first month the culture grew very slowly. From the fifth to the seventh weeks some further increase took place, and at the end of that time the growth covered the surface with a thin layer containing several large white, discrete colonies. In a 20 days' film the bacilli were uniformly stained, nearly all straight, and varied in length from 1 to 2μ.

On Broth.—In the first culture made, no growth appeared on the broth before the fifth week; a rather thin but opaque homogeneous patch then appeared, and steadily but slowly increased. A second culture also failed to yield any growth on the broth before the fifth week; floating islands then appeared and grew rather more quickly than in the former case; they were moderately dense, and exhibited some warty elevations. In a 41 days' film the bacilli measured from $\cdot 75$ to $1\cdot 5\mu$, and occasionally as much as 2μ. A few were curved and a few exhibited globular swellings.

On Glycerin-Agar.—Growth very poor and slow. At the end of two months only a delicate, semi-translucent film was formed. In a 43 days' film the bacilli resembled those grown on serum, with the exception that an occasional irregularly beaded form, about 2μ long, was found.

On Potato.—A slight amount of grey growth was obtained. It made its first appearance at the end of three weeks, and increased somewhat until the end of the second month, but never became copious or heaped up. In a 60 days' film the bacilli measured from 1 to 2 or $2\cdot 5\mu$, and were mostly straight and uniformly stained.

SUMMARY.

Growth moderately good on serum; poor on glycerinated serum; very poor on broth, glycerin-agar and potato.

(2.)

Strain.—HEIFER 231

Material Received.—Fifth generation, 15 days old, of culture isolated from Heifer 231.

On Horse Serum.—In the 6th generation (the first to be inoculated in my laboratory) a growth was just recognisable in five days ; in 14 days there was a uniform, thin, grey deposit of growth over the entire surface, studded with minute, semi-translucent elevations ; in 21 days the growth was thicker and more opaque, and the elevations were larger and definitely grey; after this period the increase was only slight. The 7th and subsequent generations grew more rapidly, a fairly good growth being obtained at the end of a week ; the growth continued for a longer period ; larger elevations were formed ; and, from the 3rd to the 4th week, the growth commenced to spread on to the surface of the glass. In a 14 days' film the bacilli were all straight and uniformly stained ; their length varied from about 2 to $\cdot 5\mu$, the average being slightly under 1μ. In a 28 days' film of the same culture there was no notable alteration in morphology.

On Bovine Serum.—The first tube inoculated happened to be taken from a poor batch of serum, and produced a scanty but uniformly distributed growth of the fine ground-glass type. Subsequent cultures, inoculated on good serum, produced an abundant growth in five days ; in less than a fortnight the surface was covered with a thick, dull layer of growth, beset with greyish yellow elevations ; growth continued for over a month, and spread on to the glass. Microscopically, the only difference noted from the horse serum cultures was that the larger forms (2–$2\cdot 5\mu$) occurred rather more frequently.

On Dog Serum.—The first culture made on this medium produced in a week an abundant, uniform, dull grey growth, without any discrete colonies. In a fortnight the growth was much increased, but remained of the same type. During the third week small, elevated, discrete, white colonies appeared. These increased in size during the fourth and fifth weeks, and developed into irregular, heaped-up, white patches. This culture, on being transferred to a second dog serum tube, yielded a more rapid and a more luxuriant growth. White, raised colonies were developed at the end of a week, and at the end of a fortnight a thick, white, warty layer covered the whole surface. A third dog serum tube, inoculated from the second, produced a growth similar to that obtained on the second. Morphologically, in 14 days' films taken from each of the three cultures, the bacilli were identical. They were all uniformly stained, nearly all straight, varied in length from $\cdot 75$ to $1\cdot 5\mu$, and averaged about 1μ.

On Glycerinated Bovine Serum.—At the end of the first week there was only a very slight increase ; on the same bovine serum, to which no glycerin had been added, and which had been inoculated simultaneously, the surface was well covered. At the end of the second week the growth was still poor, but had increased slightly, and many grey points had made their appearance ; on pure serum the growth was much better, being fairly thick, dull, and uniformly distributed. At the end of the third week there was a marked increase, the surface being fairly well covered with a greyish growth ; the amount of the growth was about equal to that obtained on pure serum. At the end of the fourth week there was a further increase and numerous dense, white colonies had appeared ; the amount of growth was quite equal to that obtained on the pure serum. There was no further increase after the fourth week. In a 20 days' film the bacilli varied from 1 to 2μ in length, were uniformly stained, and nearly all straight.

On Broth.—At the end of the 4th week several small islands of delicate semi-translucent film were floating on the broth ; between the 6th and the 7th week the surfaces of the broth became completely covered. The pellicles formed were uniform and delicate, like moistened tissue paper. In a third flask, where the layer of serum was inoculated with a particularly young and vigorous culture, small islands appeared on the broth in three weeks, and at the end of the 4th week the surface was completely covered. This pellicle was of the same general type as the others, but exhibited a few small, opaque, circular, white patches, some of them slightly raised. These white patches slowly increased in size. As regards the morphology of the bacilli, no difference was found between the last-mentioned culture, when 31 days old, and one of the earlier cultures when 66 days old. Except for the very occasional presence of a deeply stained globule, the bacilli all stained well and uniformly; none showed definite beading. The great majority of them were straight, and in the remainder the curve was only slight. Their length ranged from 1 to $1\cdot 5$ or 2μ, the majority being about $1\cdot 5\mu$.

On Glycerin-Agar.—In the first tube inoculated, minute translucent points appeared all over the surface in seven days ; in 14 days these points were more numerous, but there was no other change ; very little change took place during the next fortnight, but on the 28th day the general surface was noted to be slightly greyer than previously ; during the next three weeks the growth remained stationary and consisted merely of an extremely scanty, greyish, semi-translucent film ; about the 8th week denser grey foci appeared, and became the starting-points of a fresh growth, which took the form of circular, slowly spreading grey colonies, each with a centrally placed, raised nucleus. A control experiment behaved in essentially the same way, but the stage of slowly increasing grey, nucleated colonies was arrived at rather earlier—about the 5th week. A third tube, inoculated with a particularly and young vigorous serum culture, at first gave promise of more copious growth, the surface of the agar becoming greyish in a week ; but growth then stopped for some time, and the culture relapsed into the poorly and slowly growing condition of the former specimens. In a film from a 42 days' culture a very few bacilli stained faintly, the great majority deeply and uniformly. Most of the bacilli were short, straight, and not beaded, varying in length from $\cdot 5$ to $1\cdot 5\mu$, and generally measuring about 1μ. A few bacilli measured 2μ, and there was an occasional beaded form (about $2\cdot 5\mu$.) Curved forms were rare, and, where present, the curve was only slight.

On Potato.—Growth very slow, very scanty, and non-pigmented. In films from 48, 75, and 102 days' growths the microscopic characters of the bacilli were the same. The majority were straight or only slightly curved, uniformly stained, and from $1\cdot 5$ to 2μ long. Here and there groups were found which stained regularly, and often very faintly ; most of these bacilli were longer (from 2 to 5μ), usually curved, and contained globular bodies or irregular indications of beading.

SUMMARY.

Growth slightly better than the strain through G.P. 771.

(3.)

Strain.—G.P. 798 from GOAT 3.

Material Received. Seventh generation, 71 days old, of culture isolated from G.P. 798, which was inoculated from Goat 3.

On Horse Serum.—Formed a good layer of growth without any difficulty. In a 15 days' film the bacilli were all straight, varied in length from $\cdot 5$ to $1\cdot 25\mu$ and did not average more than $\cdot 75\mu$.

On Bovine Serum.—Growth satisfactory. In a 22 days' film the morphology was identical with that observed on horse serum.

On Dog Serum.—An abundant, dull grey growth, with small white colonies, appeared in six days. The growth steadily increased, and was copious, raised, and white at the end of a month. In a 14 days' film

the bacilli measured from ·75 to 1·25, occasionally 1·5μ, were only occasionally curved, and were all uniformly stained.

On Broth.—A thin film was formed, which covered the greater part of the surface in a fortnight. It then began to sink. At the end of a month, a fresh pellicle, of the same type as the last, began to form. In a 14 days' film the bacilli measured from 1 to 1·5μ, and were all straight and uniformly stained.

On Glycerin-Agar.—Only a very delicate, greyish film of growth was produced in six weeks. In a 42 days' film the bacilli measured from 1 to 2·5μ, and were mostly straight and uniformly stained; but there were a few curved forms and a few with globular swellings.

On Potato.—A scanty but definite growth was obtained, consisting of white streaks and numerous minute white colonies. This stage was reached at the end of the third week; subsequently very little increase took place. In a 14 days' film the bacilli measured from 1 to 3μ. The longer forms were curved, and exhibited a slight tendency to beading.

SUMMARY.

Growth similar to the strain through Heifer 231

VIRUS—H 11. E.D.
(Human Elbow Joint.)

Strain.—G.P. 918 from CALF 221.

Material Received.—Third generation, 98 days old, of culture isolated from G.P. 918, which was one of the second series of guinea-pigs inoculated from Calf 221.

On Horse Serum.—In spite of the age of the original material received, this strain grew vigorously from the first, forming a very abundant yield. In a 25 days' film the bacilli were all uniformly stained; a few of them were curved. Their length varied from 2 to ·75μ, the average being about 1μ.

On Bovine Serum.—Growth very good. In a 25 days' film the bacilli resembled those grown on horse serum.

On Broth.—Growth commenced at the beginning of the second week and the surface was covered before the end of a month. The pellicle, rather thin at first, but opaque, became thicker as the growth continued, and at the end of a month was generally very thick, wrinkled, and warty. In one flask, however, the pellicle was of a thin finely granular type, and remained stationary in this condition. In a film from a 41 days' culture some of the bacilli stained deeply, others faintly. Their length was from 1·5 to 3μ, the majority being over 2μ. About half the bacilli had a well-marked curve; from one-fourth to one-third were regularly beaded.

On Glycerin-Agar.—Growth rapid and well maintained. The surface was covered with an opaque grey layer in a week. At the end of a fortnight this layer was much denser, and numerous projecting colonies had appeared. During the three following weeks there was much further increase, and the growth became very wrinkled and warty. In a 21 days' film the bacilli exhibited great variety in shape and staining properties. Globular swellings were very frequent and regular beading was sometimes found. The length of the bacilli varied from 1 to 4μ; the shorter forms were straight and the longer generally curved.

On Potato.—Small, yellowish colonies appeared over most of the surface in a week. These continued to increase rapidly in number and size, and at the end of a month there was a thick, heaped up, yellow growth. In a 48 days' film all the bacilli, except the shortest forms, were curved; globular swellings were very numerous; regular beading was also found, but less frequently. The bacilli varied in length from 1 to about 6μ; even longer forms, possibly consisting of two bacilli joined end to end, were also seen.

SUMMARY.

Growth abundant on all media.

VIRUS—H 12. H.N.
(Human Mesenteric Glands.)

(1.)

Strain.—G.P. 577 from ORIGINAL MATERIAL.

Material Received.—Fifth generation, 46 days old, of culture isolated from G.P. 577, which was inoculated with original material.

On Horse Serum.—The first culture, inoculated from rather old material, grew poorly. Later cultures grew well. In a 25 days' film the bacilli were all uniformly stained; a few were curved. Their length varied from 2 to about ·75μ and averaged 1μ.

On Bovine Serum.—Growth abundant. In a 25 days' film the bacilli resembled those grown on horse serum.

On Dog Serum.—The first culture made on this medium grew readily all over the surface, and commenced to form opaque white colonies and patches at the end of the first week. The ultimate yield, at the end of a month, was very copious, white, and warty. A second and third generation were grown on the same medium. They both behaved like the first. Morphologically, in 14 days' films, the bacilli of the first and second generations resembled those grown on horse serum. They were uniformly stained, nearly all straight, and varied in length from ·75 to 1·5μ, exceptional forms being as short as ·5μ or as long as 2μ. In the third generation very little difference was noted, but the bacilli were, on the whole, slightly longer, some being from 2·5 to 3μ, and the average being somewhat over 1μ.

On Glycerinated Bovine Serum.—At the end of a week the surface was well covered and exhibited yellow, slightly raised colonies. During the second, third, and fourth weeks rapid increase took place, and the ultimate yield was very luxuriant, yellow, heaped up, and very warty. In a 20 days' film the bacilli measured from 1 to 3·5μ, many were curved; occasional beading and some irregular thickenings were noted.

On Broth.—A dense, white, warty, and wrinkled pellicle was formed which covered the surface in about a month's time. In a film from a 64 days' growth, the bacilli stained well and homogeneously, with the exception of an occasional globular body. None were regularly

beaded. Nearly all the bacilli had a well-marked curve. In length they ranged from 2 to $3 \cdot 5\mu$, the majority being a little over 2μ.

On Glycerin-Agar.—Grew well and rapidly. In 7 days there was a well-formed opaque layer, consisting of grey colonies massed together; in 14 days there was a copious increase, the growth being dense and studded with projecting, pear-shaped, yellowish colonies; in 21 days further increase was noted, and between the projecting colonies there was a plexus of retiform elevations; in 28 days the growth became still more wrinkled, and had spread extensively on to the glass. In a 21 days' film there was a great deal of variety in the staining properties. size, and shape of the bacilli. Length, from 1 to 4μ, the shorter forms being straight and the longer curved. Globular swellings were very frequent, and regular beading was sometimes found. Some of the bacilli stained very faintly, and had somewhat tapering ends.

On Potato.—Numerous small grey colonies appeared at the end of a week. These multiplied rapidly and gradually assumed a yellow pigment. The growth at the end of a month was abundant and heaped up. In a 25 days' film from one culture the bacilli exhibited great variety in size, shape, and staining properties, their length being from 1 to 6μ. In a film from another culture, 31 days old, the bacilli were shorter and almost all uniformly stained; their length varied from $\cdot 75$ to $3 \cdot 5\mu$, the shorter forms being straight and the longer curved.

SUMMARY.

Growth abundant on all media.

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(2).

Strain.—RABBIT 640.

Material Received.—Second generation, 23 days old, of culture isolated from Rabbit 640. This rabbit was the last of a series of rabbits in which the virus had been kept up for a period of 19½ months.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied from $\cdot 75$ to $2 \cdot 5\mu$. A few of the longer forms were curved.

On Broth.—A very dense and wrinkled pellicle was formed which covered the surface in about a month. In a 28 days' film the bacilli varied in length from 1 to $3 \cdot 5\mu$. Most of them were straight. A considerable number were beaded.

On Glycerin-Agar.—A dense, very wrinkled and warty layer was rapidly formed. In a 42 days' film the bacilli varied in length from 1 to 5μ. They were nearly all curved. Most of them were uniformly stained, but a few were beaded.

On Potato.—A brown growth appeared at the end of a week and afterwards became moderately abundant. In a 56 days' film the bacilli varied in length from 1 to 5μ. The majority were curved and beaded.

SUMMARY.

Growth luxuriant; rather better than the strain through G.P. 577 from original material.

VIRUS—H 13. A.D.

(Human Bronchial Glands and Spleen.)

(1.)

Strain.—CALF 301.

Material Received.—Third generation, 87 days old, of culture isolated from Calf 301.

On Horse Serum.—The first culture was troublesome to start, owing to the age of the material received. Later cultures grew very well. In a 20 days' film the bacilli were uniformly stained and straight. Their length varied from about 2μ to about $\cdot 75\mu$ or rather less.

On Bovine Serum.—Growth as on horse serum. In a 20 days' film the bacilli were identical with those grown on horse serum.

On Dog Serum.—Grew very vigorously, forming copious white patches in 14 days. In a 14 days' film all the bacilli were straight, and nearly all were uniformly and deeply stained, but there were a few faint forms. The average length was not much under 1μ, but very short forms, about $\cdot 5\mu$, were frequent.

On Glycerinated Bovine Serum.—At the end of a week there were numerous yellow colonies, the rest of the surface being poorly covered. A slow but general increase was maintained, and the surface gradually began to assume the appearance of frosted glass, dotted over with a number of yellow colonies. Growth continued for about seven weeks, with some increase in general density and in the size of the yellow colonies, but the total yield was only moderately abundant. In a 21 days' film the bacilli measured from 1 to 3μ, averaging about $1 \cdot 5\mu$; many of them were curved; all were uniformly stained.

On Broth.—Growth began in the second week and continued steadily but slowly, the surface not being completely covered until the end of the seventh week. The pellicle was thick, parchment-like, and wrinkled. In a 48 days' film the bacilli very frequently contained globular swellings, and a relatively small number were regularly beaded. Straight forms were more numerous than curved. The length of the bacilli varied from 1 to 4μ.

On Glycerin-Agar.—The surface was covered with a thin, opaque growth in the second week. This growth then steadily increased, assumed a frosted glass appearance, and began to creep up the sides of the glass. The ultimate yield consisted of a moderately dense layer which uniformly covered the surface but was not wrinkled or warty. In a 38 days' film the length of the bacilli ranged from 1 to 3μ; most of the longer forms were curved, some were regularly beaded, and some exhibited globular swellings. About three-fourths of the bacilli stained uniformly.

On Potato.—Fine grey colonies appeared during the second week; during the third and fourth weeks they increased and became pale yellow. The ultimate yield was not very copious. In a 33 days' film nearly all the bacilli stained uniformly, and almost all those above 1μ in length were curved. The length varied from $\cdot 75$ to about $4 \cdot 5\mu$; at least half were less than 2μ.

SUMMARY.

Growth on serum good; on glycerinated serum fairly good; on broth rather slow, but with the formation of a dense pellicle; on glycerin-agar and potato moderately good.

(2.)

Strain.—RAT 15 from CALF 301.

Material Received.—Second generation, 57 days old, of culture isolated from Rat 15 which was inoculated from Calf 301.

On Horse Serum.—Growth satisfactory. In a 21 days' film the bacilli were almost all straight, and measured from ·5 to 1·5μ in length, the average being rather less than ·75μ. They were all uniformly stained.

On Bovine Serum.—Growth as on horse serum. Morphology, in a 21 days' film, as on horse serum.

On Dog Serum.—Growth fairly good. For the first fortnight it consisted of a uniform, dull grey layer. Small white colonies appeared during the third week, and increased in size during the fourth and fifth weeks. In a 21 days' film the bacilli measured from ·5 to 1·5μ, the average being rather under 1μ. They were all straight and uniformly stained.

On Glycerinated Bovine Serum.—At the end of a week there was a very slight, irregularly distributed growth; on a control tube of the same bovine serum, without the addition of glycerin, the growth was decidedly better, the surface being uniformly covered. At the end of a fortnight the growth was still very poor, much poorer than the corresponding culture of Calf 301 of the same age; the pure serum culture was distinctly better. At the end of three weeks there was a marked increase, and many grey colonies were appearing, but the growth was still poorer than that of Calf 301; at this date the growth was about equal in amount to that obtained on pure serum. During the fourth and fifth weeks there was a slight further increase; on pure serum there was no change. The ultimate yield was a rather poor, thin growth, dotted with many fairly large, white, raised colonies; it was decidedly poorer than the corresponding culture of Calf 301. In a 21 days' film the bacilli measured from ·75 to 2μ, occasionally 2·5μ. They averaged a little over 1μ. They were all straight or only very slightly curved, and were, with the exception of a very occasional globular swelling, all uniformly stained.

On Broth.—Minute floating islands commenced to form at the end of the third week. The surface was not completely covered until the seventh week. The pellicle was thin, semi-translucent, and, in parts, speckled with small, opaque, white spots. In a 37 days' film the bacilli measured from ·75 to 1·5 and occasionally 2μ. They were straight, or only slightly curved, and uniformly stained. Forms with a slight central constriction were numerous.

On Glycerin-Agar.—Growth very poor; no more than a fine grey haze was formed. In a 40 days' film the bacilli resembled those grown on serum, with the exception of a few irregularly beaded forms 2 to 3μ long.

On Potato.—Several small grey colonies appeared in a fortnight, and increased during the third week. After this period they increased slightly, but very lowly. The total yield was very poor. In a 45 days' film the bacilli measured from 1 to 3·5μ; only a few exhibited a well-marked curve; about half were beaded, either regularly or irregularly.

SUMMARY.

On glycerinated serum poor; on broth, glycerin-agar, and potato very poor. In marked contrast to the strain from Calf 301.

(3.)

Strain.—CALF 321.

Material Received.—Sixth generation, 12 days old, of culture isolated from Calf 321.

On Horse Serum.—Good growth. In a 15 days' film the bacilli measured from ·5 to 1·25μ, and were straight and uniformly stained.

On Bovine Serum.—Good growth. In a 20 days' film, morphology of bacilli as on horse serum.

On Broth.—Growth commenced on the broth at the beginning of the third week. It increased very slowly; the surface was not quite completely covered at the end of two months; except for some patches of greater but unequal density, the pellicle was very thin, semi-translucent, and speckled with small opaque white dots. In a 34 days' film the bacilli measured from 1 to 2μ, the average being rather less than 1·5μ. They were uniformly stained and nearly all straight.

On Glycerin-Agar.—Growth very scanty. A very thin, slightly grey layer was all that was observed at the end of a month. Some pin-point grey colonies were noted at the end of seven weeks; these had increased very little in size at the end of ten weeks. In a 52 days' film the bacilli measured from ·75 to 3·5μ, averaging about 1·5μ. Most of them were straight and uniformly stained; a few were curved; an occasional bacillus was regularly beaded; no globular swellings were noted.

On Potato.—A few minute grey colonies were seen at the end of three weeks; there was no further change at the end of ten weeks. In a 71 days' film the bacilli measured from 1 to 3μ, and were straight or only slightly curved. Several were very slender and faintly stained, and several exhibited a globular swelling.

SUMMARY.

Growth very poor; similar to the strain from Rat 15.

VIRUS—H 14. F.S.

(Human Mesenteric Glands.)

(1.)

Strain.—G.P. 587 from ORIGINAL MATERIAL.

Material Received.—Eighth generation, 15 days old, of culture isolated from G.P. 587, which was inoculated with original material.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were uniformly stained and almost all straight. Their length varied from 1·5 to ·5μ, the average being slightly over ·75μ. In a 28 days' film of the same culture no change was noted.

On Bovine Serum.—Growth good. In a 14 days' film the bacilli were identical with those grown on horse serum.

On Dog Serum.—Some growth was visible in three days. At the end of a week the surface was thickly covered with a dull grey, uniform layer. This increased during the second week, but remained of the same type. White, discrete colonies appeared during the third week; and, during the fourth week, these increased in size and coalesced into irregular patches. In a 14 days' film the bacilli were straight, uniformly stained, and about ·75μ in length.

On Glycerinated Bovine Serum.—At the end of the first week there was a delicate layer of growth over the entire surface; the control tube of the same serum, without the addition of glycerin, was covered with a rather thicker layer. At the end of the second week there was a slight, fairly uniform increase; the growth on pure serum was more abundant. At the end of the

third week a marked increase had taken place, the surface being covered with a fairly thick, dull grey layer; the amount of growth was quite as great as that obtained on pure serum. At the end of the fourth week further increase was observed; the growth was dull grey and uniform on the whole, but speckled with small grey colonies; the amount of growth was quite equal to that obtained on pure serum. No further increase took place after the fourth week. In a 20 days' film the bacilli measured from ·75 to 2·5μ. averaging about 1μ. They were straight and uniformly stained.

On Broth.—Growth commenced in the second week. The rate of growth varied in different cultures; sometimes the surface was covered in a month, and in some cases growth was much slower. The type of pellicle was thin and semi-translucent; in some specimens small, grey, circular islands appeared, but in no case did any general thickening take place. In a 28 days' film most of the bacilli were short and straight; none were regularly beaded, but some exhibited a deeply stained globule. The majority measured from 1 to 1·5μ. Forms of from 2 to 3μ were also found; those longer than 2μ were generally slightly curved. On the whole, the bacilli were short, straight, and uniformly stained. In a 62 days' film from another culture no difference in morphology was noted.

On Glycerin-Agar.—A fine grey haze was formed during the first two weeks; no further visible increase took place until about the fifth week, when the growth became very slightly denser. Towards the end of the second month a few isolated, dense colonies appeared. In a 22 days' film the bacilli were uniformly stained and straight, with the exception of a few which were curved and more or less regularly beaded. The length of the bacilli varied from ·75 to 2·5μ.

On Potato.—Growth scanty and non-pigmented, ultimate yield poor. In a 50 days' film most of the bacilli were straight, uniformly stained, and varied in length from ·75 to 2μ, the average being about 1μ. Occasional groups of curved bacilli were found which contained globular swellings, and measured 2·5 to 3μ.

SUMMARY.

Growth good on serum and glycerinated serum; poor on other media.

(2.)

Strain.—CALF 125.

Material Received.—Seventh generation, 15 days old, of culture isolated from Calf 125.

On Horse Serum.—Growth satisfactory. In a 14 days' film the bacilli were straight, uniformly stained, and averaged about ·75μ in length.

On Bovine Serum.—Growth good. Morphology of bacilli, in a 28 days' film, as on horse serum.

On Dog Serum.—Growth took place rather more quickly than with the culture derived from G.P. 587, but was in other respects identical. Morphology of bacilli, in a 28 days' film, as on horse serum.

On Glycerinated Bovine Serum.—At the end of the first week the surface was only partially covered with a thin layer; at the end of the second week it was almost completely studded with semi-translucent grey points; these, at the end of the third week, had grown into discrete, greyish colonies. In the fourth week a decided increase was observed; there was no change subsequently. The ultimate yield was a rather poor growth, consisting almost entirely of small, discrete grey colonies, closely set together, and covering the greater part of the surface. In a 20 days' film the bacilli resembled those of G.P. 587 grown on the same medium.

On Broth.—Small islands were observed at the end of the third week, and at the end of the sixth week the entire surface was covered with a semi-transparent film speckled with white points. In a 33 days' film the bacilli were uniformly stained and almost all straight. They measured from ·75 to 2μ in length.

On Glycerin-Agar.—Growth very slow and poor. At the end of six weeks a very delicate, semi-transparent layer was obtained. At the end of the eighth week no further increase had taken place. In a 42 days' film the bacilli were identical, morphologically, with those grown on serum.

On Potato.—A definite, scanty, white growth was obtained at the end of the third week. Very little increase took place subsequently. In a 19 days' film most of the bacilli were straight, uniformly stained, and from 1 to 2μ in length; but there were also a few definitely beaded bacilli, some faintly stained forms, and occasional curved forms as long as 4μ.

SUMMARY.

Similar to the strain through G.P. 587.

(3.)

Strain.—G.P. 1,108 from CALF 327.

Material Received.—Third generation, 12 days old, of culture isolated from G.P. 1108, which was inoculated from Calf 327.

On Horse Serum.—Good growth obtained from the commencement. In a 15 days' film the bacilli were straight, uniform in size, uniformly stained, and did not average more than ·75μ in length.

On Bovine Serum.—Growth good. In a 20 days' film the bacilli were rather longer than on horse serum.

On Broth.—At the commencement of the fifth week small floating islands were found. These increased during the sixth and seventh weeks, forming a delicate, semi-transparent film, dotted with opaque, white spots. During the eighth week the pellicle increased in density slightly. In a 55 days' film the bacilli measured from ·75 to 1·5μ, and averaged about 1μ. They were nearly all straight or with only a slight curve, and were all uniformly stained. Forms with a slight central constriction were notable.

On Glycerin-Agar.—Growth very slow and poor. A very thin greyish film was observed at the end of a month. No further change could be seen until the end of the second month, when pin-point grey colonies were found. In a 51 days' film the bacilli measured from ·5 to 2μ, and averaged less than 1μ, the smaller forms being frequent. Only a few were curved. Some of the longer forms were regularly beaded; otherwise the staining was homogeneous.

On Potato.—Only a slight, grey growth was obtained at the end of three weeks. Very little further increase took place. In a 71 days' film the bacilli measured from 1 to 2μ. They were nearly all straight or only slightly curved, and were nearly all stained uniformly.

SUMMARY.

Similar to the two previous strains.

(4.)

Strain.—Calf 895.

Material Received.—Third generation, 8 days old, of culture isolated from Calf 895.

On Bovine and Horse Serum.—Growth good. In a 12 days' film, from bovine serum, the bacilli varied in length from 1 to 3μ, and occasionally longer forms were noted. The average length was about 1.5μ. The longer forms were often curved. Staining was uniform.

On Broth.—The surface became covered during the third week with a semi-translucent film, speckled with denser points. There was no further thickening, but during the fifth week the greater part of the growth became moist and sank. In a 22 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—A scanty, opaque layer was very slowly formed. In a 50 days' film the bacilli resembled those grown on serum.

On Potato.—During the fourth week several small grey colonies appeared. Very little further increase took place. In a 56 days' film the bacilli varied in length from about ·75 to about 3μ. They were straight, and most of them were uniformly stained, but some exhibited globular swellings.

Summary.

Growth slightly better than with the other strains of this virus.

VIRUS—H 15. I.W.

(Human Ankle Joint.)

Strain.—Calf 311.

Material Received.—Second generation, 14 days old, of culture isolated from Calf 311.

On Horse Serum.—The first culture made (third generation) developed slowly at first, but rapidly after the first fortnight, producing in a month a copious growth with large colonies. Later cultures developed more quickly. In a 19 days' film the bacilli were almost all straight and uniformly stained; their length varied from 2 to $·5\mu$, averaging rather less than 1μ.

On Bovine Serum.—Good growths were obtained without any difficulty. In a 19 days' film the bacilli were identical with those grown on horse serum.

On Broth.—Growth commenced towards the end of the second week and then developed into a white, thick, crusty film, which grew steadily but rather slowly. In a 19 days' film the bacilli stained uniformly, and were mostly from 1 to 2μ. in length. Only a few bacilli were as long as 3μ. About one-third of the bacilli were curved.

On Glycerin-Agar.—Rapid growth took place with the formation of a dense, dry, wrinkled layer. In a 31 days' film the bacilli exhibited considerable variety; short and straight forms (·5 to 1μ) were numerous, and many of them were slender and stained faintly; deeply and uniformly stained forms, measuring from 1 to $2·5\mu$, were fairly numerous, the longer of them being curved; beaded forms and forms with globular swellings were also frequent, and measured from 1·5 to 3μ. On the whole, the bacilli were notably short.

On Potato.—Grew readily and abundantly with the formation of deep yellow heaped-up colonies. In a 31 days' film the bacilli were curved, 3 to 5μ long, and often irregularly stained..

Summary.

Growth good on serum; very good on other media.

VIRUS—H 16. J.H.

(Human Knee Joint.)

(1.)

Strain.—Calf 157.

Material Received.—Fifth generation, 31 days old, of culture isolated from Calf 157.

On Horse Serum.—Growth abundant. In a 14 days' film the bacilli were nearly all straight and uniformly stained. Their length varied from 2·5 to $·5\mu$ and averaged about 1μ. In a 28 days' film of the same culture no change in morphology was noted.

On Bovine Serum.—The first and second cultures produced rather less abundant growths than were obtained on horse serum. Later cultures proved quite equal to the horse serum cultures. The bacilli in a 14 days' film were found to be slightly longer than those grown on horse serum.

On Dog Serum.—Luxuriant growth, becoming white and raised at the end of a week. In a 14 days' film the majority of the bacilli were about $1·25\mu$ long; a few were as long as $2·5\mu$. Only a few were curved; all were evenly stained.

On Glycerinated Bovine Serum.—At the end of a week large yellow colonies had developed from specks of original material; the rest of the surface was poorly covered. At the end of a fortnight the entire surface was well covered. At the end of three weeks the growth was very copious, yellow, and warty. Further increase took place during the fourth and fifth weeks, the ultimate yield being very luxuriant, deeply yellow, heaped up, and thickly studded with warty or pyriform colonies. In a 20 days' film the bacilli measured from ·75 to 3μ; a moderate number of them were curved; some had globular swellings; regularly beaded forms were occasionally present.

On Broth.—Growth was rapid, the surface being covered in from three to four weeks. The pellicle on the whole was dense and warty, though some patches were rather thinner than the rest. In a 28 days' film the bacilli were uniformly stained; a moderate number of them were curved. They varied in length from about $2·5\mu$ to about $·75\mu$, the average being $1·5\mu$.

On Glycerin-Agar.—In seven days there was a good growth, with definitely grey foci. In 14 days there was a very marked increase, with large, elevated, grey colonies. In 21 days these colonies were larger, and had become confluent over the greater part of the surface. In 28 days the surface was covered with a continuous layer of heaped-up colonies, resembling the casts of earthworms. Subsequently, no further change took place. In a 54 days' film the bacilli presented very great variety. There were short, straight, not beaded forms, measuring from ·75 to 1μ; irregularly staining forms with globular swellings ranging from 1·5 to 4μ, the longer of them being curved; slender, curved, regularly beaded forms ranging from 2·5 to 4μ; and curved, not beaded forms, measuring

from 2·5 to 3μ. The irregularly stained forms with globular swellings were the most numerous.

On Potato.—Grew readily and abundantly. Most of the surface was covered with small yellowish colonies in a week; in 14 days there was a thick, heaped-up, pale yellow growth; during the third and fourth weeks the growth steadily increased, and assumed a deeper, brownish yellow colour, In a 48 days' film great morphological diversity was found; in some clumps the bacilli were all relatively short (1 to 3μ), in others they were much longer (4 to 9μ); except the shortest forms (1 to 1·5μ) nearly all the bacilli were well curved; the longer forms were often very slender, and had a double curve; globular swellings were very numerous; regular beading occurred, but was not so common. In a film from another culture 31 days old, the only differences noted were that very long forms were not present, and regular beading was more frequent.

SUMMARY.

Growth very good on all media.

(2.)

Strain.—CALF 273.

Material Received.—Sixth generation, 12 days old, of culture isolated from Calf 273.

On Horse Serum.—Grew readily. In a 15 days' film the bacilli measured from ·75 to 1·5μ, the average being about 1μ; they were all uniformly stained and nearly all straight.

On Bovine Serum.—Growth as on horse serum. In a 19 days' film the bacilli were slightly longer than on horse serum.

On Broth.—A pellicle, moderately dense, opaque throughout, and somewhat wrinkled in patches, began to form during the second week and then slowly increased, covering nearly the whole of the surface at the end of five weeks. In a 34 days' film the bacilli measured from 1 to 2·5μ, and stained uniformly. They were nearly all straight.

On Glycerin-Agar.—Grew readily, forming in three weeks a wrinkled layer, with small, raised, white colonies. During the following four weeks the growth steadily increased, and became more markedly wrinkled and very dense. In a 28 days' film the bacilli measured from ·75 to 3μ and stained uniformly, except for a very occasional globular swelling; many of the longer forms were curved.

On Potato.—An abundant, raised, yellow growth was produced in a fortnight. The growth became markedly increased and heaped up during the following fortnight. In a 16 days' film the bacilli measured from 1 to 3·5μ, the average being about 2·5μ. Most of the longer forms were curved and nearly all of these had a central globular swelling. Many of the bacilli were rather faintly stained, and many had tapering ends.

SUMMARY.

Similar to the strain from Calf 157.

(3.)

Strain.—CALF 337.

Material Received.—Third generation, 50 days old, of culture isolated from Calf 337.

On Horse Serum.—Grew readily. In a 15 days' film the bacilli were all straight and uniformly stained, the average length being rather less than 1μ.

On Bovine Serum.—Grew readily. Morphology, in a 22 days' film, as on horse serum.

On Broth.—Growth commenced at the end of the first week, and then advanced steadily, covering nearly the whole of the surface in a month. The pellicle was only moderately dense; there were some warty patches, but the greater portion of it was thin, grey, perfectly uniform, and semi-transparent. In a 42 days' film the bacilli were uniformly stained, from 1 to 3μ long, averaging about 1.5μ, the longer forms were curved.

On Glycerin-Agar.—A slow and steady increase took place. In four weeks there were some fairly large, elevated grey masses, and the rest of the surface was covered with a thin grey layer. In six weeks there was a fairly abundant growth of the frosted glass type, without any wrinkling. In a 36 days' film the bacilli, on the whole, stained uniformly, but there were a few with globular swellings, and occasional beaded forms. Curved forms were not numerous. The length varied from ·75 to 2·5μ.

On Potato.—A slight increase took place, becoming definite at the end of the third week, and then consisting of small white colonies and thin streaks and patches. Not much increase occurred subsequently, In a 58 days' film the bacilli measured from 1 to 3·5μ. Most of them were curved; they were often regularly beaded, but more frequently irregularly, with a central thickening. The bacilli were slender.

SUMMARY.

Compared with the two previous strains, there is a marked diminution in luxuriance on broth, glycerin-agar, and potato.

(4.)

Strain.—CALF 355.

Material Received.—Twentieth generation, 27 days old, of culture isolated from Calf 355.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2·5μ and were nearly all straight and uniformly stained.

On Broth.—A moderately dense pellicle was readily formed, but soon became moist and sank. In a 21 days' film the bacilli varied in length from 1 to 2·5μ and closely resembled serum bacilli.

On Glycerin-Agar.—A thin grey layer covered the surface at the end of the first week. Subsequent increase was slow. The ultimate yield was rather scanty, on the whole, but opaque, and contained several dense patches. In a 45 days' film the bacilli measured from 1 to 3μ. They were straight and generally stained uniformly.

On Potato.—The surface became slowly covered with a white, somewhat thick, and uniformly distributed layer. In a 56 days' film the bacilli varied in length from 1 to 3·5 or 4μ. Many were curved, and beaded forms were not uncommon.

SUMMARY.

Growth similar to that of Calf 337.

(5.)

Strain.—CALF 423A.

Material Received.—Second generation, 50 days old, of culture isolated from Calf 423A.

On Horse Serum.—Grew readily and fairly copiously. In a 16 days' film the bacilli were straight, uniformly stained, and varied in length from ·75 to 1·5μ.

On Bovine Serum.—Grew readily and fairly copiously. Morphology, in a 23 days' film, as on horse serum.

On Dog Serum.—The first culture grew without any difficulty, and formed a thick, dull grey, perfectly uniform layer, without any raised white colonies or patches. A second culture produced a greyish white layer in seven days, and became more white and elevated as it increased. In a 14 days' film the bacilli were almost all straight, all uniformly stained, and measured from ·75 to 1·25μ.

On Glycerinated Bovine Serum.—At the end of the first week there was a very slight growth, which only partially covered the surface; on a control tube of the same bovine serum without glycerin the growth was distinctly better, the surface being fairly well covered. At the end of a fortnight there was some increase, but the growth was still very poor; on pure serum the growth was better. At the end of the third week there was a definite increase, but the growth was still thin, and did not exhibit any dense or discrete colonies or patches; on pure serum the surface was covered with a uniform, moderately good layer. At the end of a month a few small white colonies had appeared, and the rest of the growth, though still thin, had covered the whole surface; on pure serum the growth was better, but not abundant. No further change took place. In a 20 days' film the bacilli measured from ·75 to 2·5μ. They were straight and uniformly stained.

On Broth.—Growth commenced at the end of a fortnight, and covered the surface in from five to six weeks. The pellicle was always very delicate, semi-translucent, and speckled with occasional small, white, circular areas, which never attained a large size, nor became confluent. In a 41 days' film the bacilli were notably straight and uniform, measuring about 1μ or only slightly over.

On Glycerin-Agar.—At the end of six weeks the surface was only very slightly grey. During the third month several grey points appeared and slowly increased in size. In a 44 days' film the bacilli measured from 1 to 2·5 or 3μ. They were mostly straight and uniformly stained, but a few were curved, a few contained globular swellings, and an occasional bacillus was regularly beaded.

On Potato.—In the first three cultures made no definite growths were obtained at the end of two months.

SUMMARY.

Growth fairly good on serum; poor on glycerinated serum; very poor on broth, glycerin-agar, and potato. Growth very much poorer than that of the strain through Calf 337.

(6.)

Strain.—CALF 559.

Material Received.—Primary culture, 12 days old, from Calf 559, which had been inoculated with a culture from Calf 423A.

On Dog and Bovine Serum.—Good growths were obtained from the commencement. On dog serum, in a 13 days' film, the bacilli measured from ·75 (occasionally ·5) to 1·5μ, and were all straight and uniformly stained. On bovine serum, in a 26 days' film, the bacilli were slightly longer and were occasionally curved.

On Broth.—A pellicle was formed which covered the surface in rather less than five weeks, and then began to become moist and fall to the bottom. The pellicle was grey, moderately thin, but opaque, and speckled with several warty elevations. In a 20 days' film the bacilli measured from 1 to 2μ, and were rarely as long as 2·5μ. They were uniformly stained and straight or only very slightly curved.

On Glycerin-Agar.—Growth commenced slowly, assuming a frosted glass appearance during the third week, with small grey colonies dotted over the general surface of the growth. These grey colonies became denser and somewhat warty during the fifth and sixth weeks. The total yield was only moderately good. In a 42 days' film the bacilli varied in length from 1 to 2·5 or 3μ, the longer forms being rare. The majority were straight, and about half were uniformly stained. The rest were stained irregularly and often contained globular swellings.

On Potato.—A definite, but scanty, grey growth was visible at the end of a fortnight. The yield never became abundant. At the end of seven weeks the surface was covered with a grey, rather thin, moderately uniform growth, slightly raised in patches. In a 42 days' film the bacilli measured from 1 or 1·5 to 2·5μ, occasionally 3μ. Exceptional forms were found measuring ·75μ or even less. Almost all the bacilli were straight. About three-fourths of them were uniformly stained; the rest were either stained faintly, or occasionally, regularly beaded.

SUMMARY.

About equal to, or slightly better than, the growth obtained from the strain through Calf 337. The advance in luxuriance compared with the Calf 423A strain is noticeable.

VIRUS—H 17. Sp. B.

(Mixed Human Sputum.)

(1.)

Strain.—CALF 265.

Material Received.—Third generation, 15 days old, of culture isolated from Calf 265.

On Horse Serum.—Growth commenced about the fifth day and was copious at the end of three weeks. In a 14 days' film the bacilli were all uniformly stained; a moderate number were curved. Their length varied from about 2 to about ·75μ, the average being 1μ. In a 28 days' film from the same culture the bacilli were slightly shorter and a few beaded forms were noted. Short, straight, forms with a slight central constriction were frequently observed.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli averaged about 1μ in length; a few forms as long as 3μ were noted; a few bacilli contained a deeply stained, globular deposit; forms with a slight central constriction were present, but not numerous.

On Dog Serum.—An abundant, white, heaped-up growth was produced. The culture began to exhibit white colonies and patches at the end of the first week. In a 14 days' film the bacilli resembled those grown on bovine serum, but curved forms and forms as long as 3μ were rather more numerous.

On Glycerinated Bovine Serum.—At the end of a week yellow colonies had developed from particles of original material, but there was not much growth on the rest of the surface; on the control tube of the same sample of bovine serum, without the addition of glycerin, there were similar yellow colonies, and the rest of the surface was covered with a uniform layer of growth. At the end of a fortnight in addition to large, projecting, yellow colonies, the surface was well covered with a uniform layer; the growth on pure bovine serum was quite as good, and was of the same type. At the end of three weeks both cultures showed further increase, but the growth on glycerinated serum was the better of the two. At the end of four weeks the growth on glycerinated serum was distinctly the rather more copious of the two; the growth on pure serum was much less moist. The ultimate yield was a fairly thick, heaped-up, yellow growth. In a 21 days' film the bacilli measured from 1 to 5μ; many were curved, and many were beaded.

On Broth.—A thick, warty, and crusty pellicle covered the surface in less than five weeks. In films from a 41 days' culture, most of the bacilli were curved and measured from 2·5 to 3μ, Short and straight forms, about 1μ in length, were also present. None of the bacilli were regularly beaded; some of them contained deeply stained globules.

On Glycerin-Agar.—Growth rapid and abundant, producing in a month a thick, dry, wrinkled layer with many projecting colonies. In films from 42 days' and 65 days' growths, the majority of the bacilli were about 2μ in length, evenly and deeply stained, not beaded, and generally curved. There were also longer and shorter forms, some beaded and some with globular swellings, but on the whole the character of the bacilli was uniform.

On Potato.—Growth rapid and luxuriant, producing an abundant, yellow, heaped-up, crumb-like layer in 14 days. In a 19 days' film, every morphological variety of tubercle bacillus was to be found. The length ranged from ·75 to 8μ; long, short, and intermediate forms being all about equally represented. All the forms above about 2·5μ were curved. Both regularly beaded forms and forms with deeply staining globular bodies were numerous.

SUMMARY.

Growth good on serum and luxuriant on other media.

(2.)

Strain.—CALF 339.

Material Received.—Fourteenth generation, 14 days old, of culture isolated from Calf 339.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ and were straight and uniformly stained.

On Broth.—A thick pellicle was formed which covered the surface in from three to four weeks, and showed no tendency to sink. In a 22 days' film the majority of the bacilli resembled those grown on serum, but some rather longer forms were present.

On Glycerin-Agar.—In 12 days the surface was well covered with a grey, slightly wrinkled layer. The growth afterwards became much denser, very wrinkled, and spread on to the glass. In a 42 days' film the bacilli varied in length from ·75 to 3·5 and occasionally 4·5μ. Curved forms were frequent; irregularly stained forms were less frequent, but not uncommon.

On Potato.—The surface was well covered with yellow colonies in less than a fortnight. The growth became moderately abundant at the end of a month and then ceased. In a 56 days' film the bacilli varied in length from ·75 to 5μ. Most of the longer forms were curved. Many bacilli were regularly beaded and many were stained irregularly.

SUMMARY.

Growth good on all media, but less luxuriant than the Calf 265 strain.

(3.)

Strain.—RABBIT 181 from CALF 539.

Material Received.—Third generation, 58 days old, of culture isolated from Rabbit 181 which was inoculated from Calf 539.

On Dog and Bovine Serum.—Growth good. On bovine serum, in a 21 days' film, the bacilli were straight and uniformly stained; forms as short as ·5μ were frequent and the length varied from ·5 to about 1·25μ. On dog serum, in a 28 days' film, the only difference observed was that forms as long as 1·5 and 2μ were occasionally found.

On Broth.—The surface was covered at the beginning of the third week with a film of moderate thickness. The pellicle afterwards became denser and markedly wrinkled. In a 25 days' film the bacilli measured from 1 to 2·5μ. most of the bacilli were straight and uniformly stained; forms with slight central constrictions were not infrequent; a slight tendency to beading was occasionally observed.

On Glycerin-Agar.—During the second week the surface became moderately well covered with a grey layer. During the third week the growth assumed the frosted glass type, and small, yellow, raised points appeared. The growth then steadily progressed, became wrinkled during the sixth week and definitely warty during the seventh week. In a 42 days' film the bacilli varied in length from 1 to 3 or 3·5μ. The longer forms were generally curved. Regularly beaded forms were moderately frequent; the rest were uniformly stained.

On Potato.—The growth was slow during the first fortnight; at the end of this time many small, pale yellow colonies had appeared. The growth then steadily increased, becoming moderately abundant and warty at the end of six weeks. In a 27 days' film the bacilli measured from 1 to 3μ and occasional longer forms were noted. Two-thirds or more were straight; except for a slight tendeney to beading amongst some of the bacilli, the staining was uniform.

SUMMARY.

Growth fairly good on all media, but not so good as the strain from Calf 265.

(4.)

Strain.—G.P. 1542 from Calf 553.

Material Received.—Eighth generation, 20 days old, of culture isolated from G.P. 1542, which had been inoculated from Calf 553.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·5 to 1·5μ, and averaged about ·75μ. They were straight and uniformly stained.

On Broth.—A thin, partially transparent layer covered the surface in a month. The pellicle showed no tendency to thicken. In a 22 days' film the bacilli resembled those grown on serum.

On Glycerin-Agar.—At the end of three weeks no more than a fine grey haze was formed. At the end of two months very little increase had taken place, but there were a few opaque grey colonies. In a 42 days' film the bacilli varied in length from 1 to 3μ. They were, on the whole, straight and uniformly stained, but some of the longer forms were curved and some bacilli contained globular swellings.

On Potato.—The growth was so scanty as to be hardly visible to the naked eye. In a 59 days' film there was definite evidence of growth. The bacilli varied in length from 1 to 4·5μ. They were uniformly stained. The longer forms were curved.

Summary.

Growth very poor, but rather better than the strains from Calves 555 and 571.

(5.)

Strain.—Calf 555.

Material Received.—Primary culture, 42 days old, isolated from Calf 555.

On Dog and Bovine Serum.—The first culture made on dog serum from the material received showed a definite growth in 5 days and was fairly good in a fortnight. The first culture on bovine serum, also made from the material received, showed a rather poorer growth than the dog serum tube. Later generations grew well on serum. On dog serum, in a 14 days' film, the bacilli measured from ·75 to 1·5μ, occasionally 2μ, and were straight and uniformly stained. On bovine serum, in a 21 days' film, the morphological characters of the bacilli were the same as on dog serum.

On Broth.—A few small, delicate, nearly translucent islands appeared at the end of the third week. The pellicle extended very slowly and covered the greater part of the surface in six weeks. The growth remained very delicate and semi-translucent, speckled with some small, grey, nodular points. In a 29 days' film the bacilli measured from 1 to 2μ. They were all uniformly stained and nearly all straight.

On Glycerin-Agar.—Growth extremely poor, only a faint grey haze being formed at the end of eight weeks. In a 56 days' film the bacilli measured from ·75 to 3μ, the greater number being under 1·5μ. The shorter forms were straight and uniformly stained; the longer forms were often curved, stained irregularly, or regularly beaded.

On Potato.—There was no visible evidence of growth in six weeks, and microscopic examination showed that the bacilli present were scanty and morphologically identical with those grown on serum.

Summary.

Growth fairly good on serum; very poor on other media. A marked contrast to strains (1) and (2).

(6.)

Strain.—Calf 571.

Material Received.—Primary culture, 57 days old, isolated from Calf 571.

On Dog and Bovine Serum.—The first culture made on dog serum from the material received showed a definite growth in five days and produced a good yield in a fortnight. The first culture on bovine serum was poorer, but subsequent cultures were good. On dog serum, in a 14 days' film, most of the bacilli were from ·75 to 1μ in length, neither shorter nor longer forms being frequent. They were all straight and uniformly stained. On bovine serum, in a 20 days' film, the bacilli were slightly longer.

On Broth.—Many delicate semi-translucent, speckled islands made their appearance during the third week, and extension then took place with moderate rapidity, the greater part of the surface being covered in five weeks. The pellicle did not become any thicker. In a 29 days' film, the bacilli were from 1 to 2μ in length, uniformly stained, and occasionally curved.

On Glycerin-Agar.—A thin grey haze was all that was visible at the end of eight weeks. In a 56 days' film the bacilli varied in length from ·75 to 2·5μ. A small number of curved, or beaded, or irregularly stained forms were present, but the great majority were straight, uniformly stained, and less than 2μ in length.

On Potato. There was no definitely visible evidence of growth at the end of six weeks. Microscopic examination of a 29 days' film showed that bacilli were rather plentiful and that a relatively small number of them were longer than those grown on serum and sometimes regularly beaded.

Summary.

Similar to the strain through Calf 555.

VIRUS—H 18. T.T.

(Human Mesenteric Glands.)

(1.)

Strain.—G.P. 999 from Calf 131.

Material Received.—Third generation, 72 days old, of culture isolated from G.P. 999, one of the third series of guinea-pigs inoculated from Calf 131.

On Horse Serum.—In spite of the age of the original material, cultures grew very well from the commencement. In a 25 days' film the bacilli were uniformly stained and nearly all straight. Their length varied from 2 to about ·75μ and averaged rather over 1μ.

On Bovine Serum.—Growth as on horse serum. Morphology of bacilli, in a 25 days' film, as on horse serum.

On Dog Serum.—Grew vigorously, forming a white, warty, wrinkled growth in 14 days. In a 14 days' film most of the bacilli were straight, uniformly stained, and averaged under 1μ in length. A few longer, curved, not beaded forms, measuring up to 2·5μ, were present. There were also a few bacilli with globular swellings.

On Glycerinated Bovine Serum.—The growth was moderately good during the first three weeks; at the end of three weeks several small, raised, yellow colonies were appearing. From the fourth to the seventh weeks a slow but steady increase was maintained, and the ultimate yield was fairly luxuriant, yellow, and heaped up. In a 21 days' film the bacilli measured from 1 to 3μ; a moderate number were curved; with the exception of an occasional beaded form they were all uniformly stained.

On Broth.—Pellicles covering the surface were produced without any difficulty in a month, but were generally of unequal density, like irregular patches of parchment, thinning out into tissue paper. In a film from a 41 days' growth the majority of the bacilli were curved, uniformly stained, and about 3μ in length.

On Glycerin-Agar.—Grew readily, forming in a month an abundant, fairly uniform, dry, very wrinkled growth, without many large projecting colonies. No further increase took place. In a 21 days' film the bacilli were rather long (from 2·5 to 4μ). Most of the bacilli were curved. Both regular beading and globular bodies occurred occasionally, but the majority of the bacilli did not stain unequally. Some of them stained faintly.

On Potato.—Grew readily at first, with the production of a brownish-yellow layer in less than three weeks. Subsequently, very little further increase took place. In a 25 days' film the bacilli varied in length from ·75 to 4μ. The shorter and the longer forms were about equally numerous; about half the bacilli were straight. Globular swellings were numerous; no regular beading was observed.

SUMMARY.

Growth good, on the whole, but with some tendency to fall off, after making a vigorous start.

(2.)

Strain.—CALF 405.

Material Received.—Second generation, 77 days old, of culture isolated from Calf 405.

On Horse Serum.—Satisfactory growth was obtained without any difficulty. In a 15 days' film the bacilli measured from ·75 to $1·5\mu$, averaging about 1μ, and were almost all straight.

On Bovine Serum.—Growth as on horse serum. In a 22 days' film the bacilli were identical with those grown on horse serum.

On Dog Serum.—A fairly thick, grey, uniform layer was formed in five days; at the end of a fortnight a copious, white, heaped-up growth was present; and further increase took place during the third and fourth weeks. In a 15 days' film the bacilli measured from ·75 to $1·5\mu$, were uniformly stained, and rarely curved.

On Broth.—Grey, lacey patches over an inch in diameter were formed in a fortnight. In three weeks most of the surface was covered, and some warty thickenings were present. The pellicle ultimately formed was only moderately thick, and was speckled with white raised points. In a 34 days' film the bacilli measured from 1 to $3·5\mu$, the average being rather less than 2μ. The longer forms were often curved, and some of them were more or less regularly beaded.

On Glycerin-Agar.—Grew well, forming, in from four to five weeks, a moderately abundant wrinkled layer, with projecting yellowish colonies. In a 31 days' film the bacilli measured from 1 to 3μ; several of the longer forms were curved; with the exception of an occasional globular swelling all the bacilli stained uniformly.

On Potato.—At the end of a fortnight there were numerous, fairly large, raised, yellowish colonies. At the end of three weeks the growth was fairly copious, somewhat heaped up, and was of a yellow colour. Some further increase was observed at the end of five weeks, but no further growth took place after that period. In a 37 days' film the bacilli measured from ·75 to 5μ; many were from 2·5 to 3μ long; short forms, from ·75 to 1μ, were much more numerous than forms over 3μ. About three-fourths of the bacilli were beaded, and the majority of these exhibited regular, discrete beads About two-thirds of the bacilli were curved.

SUMMARY.

The amount of growth was about equal to that obtained with the previous strain.

VIRUS—H 19. S.W.

(Human Mesenteric Glands.)

(1.)

Strain.—HEIFER 239.

Material Received.—Fifth generation, 12 days old, of culture isolated from Heifer 239.

On Horse Serum.—Grew readily. In a 15 days' film the bacilli measured from ·75 to $1·5\mu$, and were straight and uniformly stained.

On Bovine Serum.—Growth as on horse serum. In a 20 days' film the bacilli were rather shorter than on horse serum.

On Broth.—Large patches of thin, greyish, homogeneous film were formed at the end of a fortnight, and the entire surface was covered in a month. The film always remained very delicate, and readily broke up into islands, which had a tendency to curl up at the edges and sink. In a 34 days' film the bacilli exhibited a great deal of variety in size and shape. They varied from 1 to 4μ in length; only the shortest forms were deeply and uniformly stained; of the rest, many were regularly beaded, many stained irregularly and somewhat faintly, many contained globular swellings, and about one-third were curved.

On Glycerin-Agar.—Grew slowly but uninterruptedly, forming at the end of a month a thin layer, commencing to assume the frosted-glass type. This layer subsequently became rather denser, but was never very copious nor wrinkled. In a 41 days' film the bacilli exhibited very great variety. They measured from 1 to 4μ. Most of the longer forms were curved. Very few, except the shortest forms, were uniformly stained. Regular beading, globular swellings, thickened extremities, faintly stained forms, and forms with tapering ends were all of frequent occurrence.

On Potato.—A slight increase was observed during the second week. At the end of the third week numerous minute grey colonies were formed. After this period the growth increased very slowly for two or three weeks; it never became copious. In a 51 days' film the bacilli measured from 1 to $2·5\mu$, averaging 1.5μ or rather less. There was a very large number of short, straight forms, with a well-marked central constriction. There were a few regularly beaded forms. A few of the longer forms were curved.

SUMMARY.

Growth good on serum; poor on broth; fair on glycerin-agar; poor on potato.

(2).

Strain.—GOAT 11.

Material Received.—Third generation, 31 days old, of culture isolated from Goat 11.

On Horse Serum.—Growth rather troublesome at first, afterwards fairly good. In a 14 days' film the bacilli were all uniformly stained; a few were curved. Their length varied from 2 to ·5μ, the average being less than 1μ. In a 28 days' film from the same culture curved forms were rather more frequent.

On Bovine Serum.—Growth a little capricious at first, but satisfactory when transplanted from young cultures. In a 14 days' film the bacilli resembled those grown on the corresponding tube of horse serum.

On Dog Serum.—At the end of a fortnight the growth, though good, was of the dull, uniformly grey type, without any discrete colonies. During the third week it became more opaque, and during the fourth week white, raised patches appeared. In a 14 days' film the bacilli were mostly from ·75 to 1·25μ long; exceptional forms measured from 1·5 to 2μ; the average was rather less than 1μ. All were uniformly stained; a few only were curved.

On Glycerinated Bovine Serum.—At the end of a week the surface was covered with a dull, uniform, abundant growth; the growth on the same bovine serum, to which no glycerin had been added, was about the same in character and amount. At the end of a fortnight the growth had become greyish and was slightly heaped up; on pure bovine serum the yield was less in amount, though fairly good. There was some further increase at the end of the third week; the growth on pure serum was more uniform. At the end of a month the growth had become definitely grey, and was dotted with white colonies; the growth on pure serum was much drier and distinctly less copious. At the end of the seventh week some little further increase was noted and the growth was very moist, white, and moderately abundant. In a 21 days' film the bacilli measured from 1 to 5μ, and exhibited great variety in form. Many were curved; many were regularly beaded; many had globular swellings, some stained faintly.

On Broth.—The growth was fairly rapid, but variable in the type of pellicle produced. In one flask the pellicle was thin, homogeneous, at first semi-transparent and afterwards opaque, but never became thick or elevated. Other flasks, probably owing to their being inoculated with more vigorous material, produced a tougher pellicle, thickly and uniformly studded with warty elevations, though the intervening portions were much thinner. The morphology of the bacilli also proved to be inconstant. In one film, taken from a 75 days' culture of the thinner type, the bacilli were uniformly short, straight, and not beaded, measuring from 1 to 1·5μ. In another film, taken from a 21 days' culture of the more luxuriant type, the bacilli showed great irregularity io size, shape, and staining properties. A very large number contained deeply staining globular bodies, and there were also some regularly beaded forms. Some of the bacilli were very slender and stained faintly. The length varied from 1 to 5 or even 6μ, the longer forms (over 3μ) preponderating. Most of the longer forms had a well marked curve.

On Glycerin-Agar.—A delicate, but opaque layer was formed during the first fortnight. This gradually increased, and during the second month formed several irregular, raised, dense patches, the rest of the layer remaining thin. In a 71 days' film the bacilli were notably short, averaging less than 1·5μ, whilst many were even less than 1μ. Most of them were straight and regularly stained. No beading or globular bodies were found. The characters of the bacilli were strikingly uniform.

On Potato.—Growth slow and scanty; white at first, but pigmented after the first month; the yield never became copious. In a 33 days' film the bacilli were often curved, sometimes exhibited globular swellings, and were frequently regularly beaded. Their length varied from 1 to 4·5μ.

SUMMARY.

Growth fairly good on serum, better on glycerinated serum; on broth somewhat poor and uncertain; on glycerin-agar and potato rather poor. Growth on the whole perhaps a little better than the strain through Heifer 239.

VIRUS—H 20. F.L.

(Human Mesenteric Gland.)

Strain.—CALF 213.

Material Received.—Fourth generation, 20 days old, of culture isolated from Calf 213.

On Horse Serum.—Growth good. In a 26 days' film the bacilli were straight and uniformly stained. Their length varied from 1·5 to ·5μ, averaging not much over ·75μ. Forms with a slight central constriction were frequent.

On Bovine Serum.—Growth as on horse serum. In a 26 days' film the bacilli resembled those grown on horse serum.

On Dog Serum.—Grew well, but did not develop raised, white colonies until the commencement of the third week. In a 15 days' film the bacilli were notably short, straight, and uniform in every respect; the average length was not more than ·75μ, and very few were over 1·25μ.

On Glycerinated Bovine Serum.—The surface was covered with a thin layer of growth in a week; the control tube of the same bovine serum, without glycerin, showed a rather better growth. At the end of a fortnight the growth was thicker, greyish, and uniform; the growth on pure serum was not quite so good. At the end of three weeks there was some further increase, and white raised colonies had begun to appear; the growth on pure serum was about equal in amount, but more uniformly distributed. At the end of four weeks there was some little further increase; on pure serum the growth was drier, more uniform, and rather thinner. No further change took place. The ultimate yield was moist, white, irregularly distributed, and rather scanty. In a 21 days' film the bacilli varied in length from 1 to 5μ. Many were curved, and many were regularly beaded; curved, regularly beaded forms from 4 to 5μ long were frequent. Globular swellings were numerous, and some bacilli were faintly or irregularly stained.

On Broth.—The bacilli began to grow on to the broth about the end of the first week, and then rapidly spread, the entire surface and part of the glass being covered at the end of the fourth week. The pellicle resembled fine, semi-transparent gauze, upon which small, raised, white spots were abundantly and uniformly distributed. In a film from a 25 days' growth there was great irregularity in the size, shape, and staining properties of the bacilli. Globular bodies were very numerous, and a few regularly beaded forms occurred. Curved forms were quite as numerous as straight. Some bacilli were over 4μ in length; the majority were from 2 to 3μ.

On Glycerin-Agar.—Growth slow and patchy. Some fairly dense grey patches and some raised colonies were formed, but the rest of the surface consisted of a thin, opaque layer. In a 29 days' film the bacilli presented marked irregularity. Shadowy forms and globular swellings were numerous. At least half the bacilli were curved, and many had a tendency to regular beading. The length varied from 1·5 to 4·5μ.

On Potato.—A scanty, non-pigmented growth appeared during the third week and then progressed slowly, but never became abundant. In a 33 days' film the bacilli were for the most part straight and uniformly stained, but some were curved and beaded. Their length varied from ·75 to 3μ.

SUMMARY.

Growth good on serum, but somewhat poor on glycerinated serum; on broth, thin, on glycerin-agar and potato rather poor.

VIRUS—H 22. F.W.

(Human Lung and Bronchial Glands.)

Strain.—CALF 293.

Material Received.—Third generation, 15 days old, of culture isolated from Calf 293.

On Horse Serum.—Growth good. In a 14 days' film the bacilli were uniformly stained and almost all straight. Their length varied from 2 to ·5μ, the average being less than 1μ. In a 28 days' film from the same culture no change was noted.

On Bovine Serum.—The first two or three cultures, on excellent serum, grew rather reluctantly at first; increase took place from the specks of original material, and gradually led to the formation of raised, yellow, isolated colonies, the intervening space exhibiting hardly any growth. After about the third week the growth became more vigorous, and began to spread all over the surface; rubbing up the surface with the plantinum loop hastened this increase of vigour. Later cultures, however, grew from the first as a thick, uniform, opaque layer, amongst which raised yellow colonies made their appearance on about the fourteenth day, and subsequently became large and numerous. In a 14 days' film the bacilli were identical with those grown on horse serum.

On Broth.—Small floating islands were found in a week, and in about three weeks the growth had covered the entire surface, and was creeping up the glass. The pellicle was thick and opaque, and grew thicker as it became older; it exhibited irregular patches of greater thickness than the rest, and white warty elevations. In a 25 days' film the bacilli were uniformly stained; some of them were curved. Their length varied from 1 to 2·5μ.

On Glycerin-Agar.—In seven days the surface was covered uniformly with a grey growth, exhibiting many raised points; in 14 days the growth was much increased; in 21 days it had extended on to the glass, and numerous warty, yellowish colonies projected from the surface of the agar; in 28 days there was a thick, dry, wrinkled growth with numerous yellow, warty, or pear-shaped projections. Morphologically, in a 42 days' growth, the characters of the bacilli were varied. Equally numerous with short, straight, not beaded forms, were curved, irregularly staining forms, from 2 to 3μ in length, exhibiting globular swellings.

On Potato.—Good growths were obtained without any difficulty. On a good potato there were found definite signs of growth in five days; in eight days numerous yellowish colonies appeared, the largest about ½ mm. in diameter; in 14 days the colonies had increased in size and the growth had spread over the greater part of the surface, and was of a definitely yellow colour; in 21 days the growth was becoming heaped up; and in 28 days there was a further increase. In a 31 days' film nearly all the bacilli were slender, and had a well-marked curve, often a double curve; they varied in length from 3 to 7μ the average being between 4 and 5μ; there were indications of regular beading in many of these bacilli, but completely discrete beading was not frequent; a few of them had a globular thickening, centrally placed. There were also present a small number of short, rather thick, uniformly stained, and generally straight forms, measuring from 1 to 2μ.

SUMMARY.

Growth good on serum and abundant on other media.

VIRUS—H 23. J.P.

(Human Lung.)

Strain.—CALF 365.

Material Received.—Primary culture, 57 days old, isolated from Calf 365.

On Horse Serum.—On the first subculture very little growth took place during the first week, and a scum formed on the surface; beneath this scum minute colonies could be seen, and round some of them there was a moist zone from which the scum had disappeared. At the end of the second week the scum had entirely disappeared, and the surface was covered with a copious growth, studded with larger grey colonies. All later cultures grew rapidly and luxuriantly. In a 20 days' film beaded or curved forms were rare. A large number of the bacilli were very short, less than ·75μ. Forms above 1μ in length were comparatively rare.

On Bovine Serum.—Both the first subculture and all later cultures grew vigorously and rapidly from the commencement. From a 20 days' growth the bacilli were found to be rather longer than on horse serum, the average length being slightly under 1μ.

On Dog Serum.—The first culture made on this medium grew readily all over the surface, and commenced to form opaque white colonies and patches at the end of the first week. The ultimate yield, at the end of a month, was very copious, white, and warty. A second and third generation were grown on the same medium. They both behaved like the first. Morphologically, in 14 days' films, the bacilli of the first and second generations resembled those grown on horse serum. They were uniformly stained, nearly all straight, and varied in length from ·75μ to 1·5μ, exceptional forms being as short as ·5μ, or as long as 2μ. In the third generation very little difference was noted, but the bacilli were, on the whole, slightly longer, some being from 2·5 to 3μ, and the average being somewhat over 1μ.

On Glycerinated Bovine Serum.—At the end of a week the surface was well covered and exhibited yellow, slightly raised colonies. During the second, third, and fourth weeks rapid increase took place, and the ultimate yield was very luxuriant, yellow, heaped up, and very warty. In a 20 days' film the bacilli measured from 1 to 3·5μ; many were curved; occasional beading and some irregular thickenings were noted.

On Broth.—Thick, irregular, warty pellicle. In a film from a 39 days' culture, the length of the bacilli varied from 1·5 to 3μ; straight forms were more numerous than curved, and short forms were more frequent than long. An occasional bacillus was beaded or exhibited a globular swelling.

On Glycerin-Agar.—Growth very vigorous, forming a dense, wrinkled growth, with numerous projecting, polypoid colonies. In a film from a 39 days' growth nearly all the bacilli were curved and beaded, the beading being generally uniform, though forms with globular swellings were also present. The bacilli were mostly from 2 to 4μ long; several measured 5μ.

On Potato.—A copious, yellow, heaped up growth was readily formed. In a 31 days' film the majority of the bacilli were slender, curved, and partially beaded; their length varied from 3 to 6μ. Short, straight forms (1 to 2μ), were also present.

SUMMARY.

Growth very good on all media.

VIRUS—H 25. A.T.

(1.)

Strain.—G. P. 1282 from Original Material.

Material Received.—Third generation, 14 days old, of culture isolated from G.P. 1282.

On Horse Serum.—Vigorous growth. In a 12 days' film the bacilli were uniformly stained; a few were curved. Their length varied from 2μ to rather less than $\cdot 75\mu$, the average being about 1μ.

On Bovine Serum.—Growth as on horse serum. In a 14 days' film the bacilli were identical with those found on horse serum.

On Broth.—Growth began as a rather thin, opaque pellicle, mottled with some denser patches, none of which were crusty or warty. The pellicle grew rapidly, and when about a month old became much denser and spread over the glass. In a 28 days' film a large proportion of the bacilli were curved and regularly beaded, measuring from $2\cdot 5$ to $3\cdot 5\mu$.

On Glycerin-Agar.—Growth rapid, with the formation of a dense, very wrinkled layer. In a 10 days' film the majority of the bacilli were about 2μ in length, uniformly stained, and generally curved. Some longer and some shorter forms also occurred. Beaded, or irregularly stained forms were present, but relatively rare.

On Potato.—Growth rapid. Raised, crumb-like, yellow colonies appeared in seven days, and at the end of a fortnight there was a copious increase and the colonies had acquired a brownish tinge. Some further increase, but at a slower rate, took place. In a 12 days' film, with the exception of an occasional long, curved form, the bacilli resembled those grown on serum; they measured from $\cdot 75$ to $1\cdot 5$ or 2μ, and were nearly all straight.

Summary.

Growth very good on the whole, but somewhat variable on broth.

(2).

Strain.—Chimpanzee 1.

Material Received.—Second generation, 9 days old, of culture isolated from the liver of Chimpanzee 1.

On Horse Serum.—Growth good. In a 12 days' film the bacilli varied in length from 1 to $2\cdot 5\mu$ and averaged over 1μ. With the exception of a few which showed globular swellings, they were straight and uniformly stained.

On Broth.—The surface was covered in about a month with a pellicle which was very thick in parts but not of equal density. In a 21 days' film the bacilli varied in length from 1 to 3μ. They were uniformly stained. Less than half were curved.

On Glycerin-Agar.—The first three cultures grew poorly, but the fourth tube which was inoculated from serum gave an abundant growth which was very wrinkled and very warty during the fourth week and became denser afterwards. In a 44 days' film the bacilli varied in length from 1 to 4μ, and were uniformly stained, the great majority were curved.

On Potato.—An abundant, deep brown layer was readily formed. In a 56 days' film the bacilli varied in length from 1 to 5 or occasionally 6μ. The great majority were curved. Most of them were uniformly stained but a tendency to beading was noted in the longer forms.

Summary.

Growth very good on the whole, but gave some trouble at first on glycerin-agar. Not quite equal to the strain, through G.P. 1282, of original material.

(3).

Strain.—Chimpanzee 3.

Material Received.—Second generation, 9 days old, of culture isolated from the bronchial gland of Chimpanzee 3.

On Horse Serum.—Growth good. In a 12 days' film the bacilli varied in length from $\cdot 75$ to $2\cdot 5\mu$ and averaged slightly over 1μ. They were nearly all straight and uniformly stained.

On Broth.—Growth commenced during the beginning of the second week and the surface was well covered at the end of a month. The pellicle was very thick and wrinkled. In a 33 days' film the bacilli varied in length from $\cdot 75$ to 3μ. The great majority were straight. About one third were irregularly stained.

On Glycerin-Agar.—Growth was very abundant and rapid; a thick, warty, very wrinkled layer was formed as early as the second week, and the ultimate yield was very copious. In a 44 days' film the bacilli varied in length from 1 to $3\cdot 5\mu$ and were uniformly stained. About half were curved.

On Potato.—A moderately thick, brown layer was formed during the first six weeks. In a 56 days' film the bacilli varied in length from $\cdot 75$ to 6 or 7μ. Long forms (5 to 6μ) were very numerous. The great majority of the bacilli were curved and either regularly beaded or with a tendency to beading.

Summary.

Luxuriant. Rather better than culture of original material isolated through G.P. 1282.

(4).

Strain.—G.P. 1833 from Dog 1.

Material Received.—Third generation, 23 days old, of culture isolated from G.P. 1833, which had been inoculated from Dog 1.

On Horse Serum.—Growth poor at first, afterwards good. In a 14 days' film the bacilli varied in length from $\cdot 75$ to $2\cdot 5\mu$, averaging slightly over 1μ. Some of the longer forms were curved and a few were beaded.

On Broth.—Growth took place slowly, but a thick, tough pellicle was gradually formed. In a 27 days' film the bacilli varied in length from 1 to 4μ. Curved forms were numerous, and regular beading was common.

On Glycerin-Agar.—A thick, wrinkled, and warty pellicle was formed, though growth was rather slow during the first fortnight. In a 52 days' film the bacilli

varied in length from $1 \cdot 5$ to 4μ. Most of them were curved and uniformly stained.

On Potato.—Growth was fairly abundant. It first consisted of yellow colonies. Afterwards, these coalesced and assumed a brownish pigment. In a 56 days' film the bacilli varied in length from 1 to 6μ, and most of them were curved. The majority were regularly beaded, but globular thickenings were not uncommon.

SUMMARY.

Growth abundant, but perhaps not quite so good as the original strain of this virus.

VIRUS—H 26. K.M.
(Human Kidney.)

Strain.—G. P. 1326 from ORIGINAL MATERIAL.

Material Received.—Second generation, 57 days old, of culture isolated from G. P. 1326.

On Horse Serum.—Growth satisfactory. In a 15 days' film the bacilli measured from $\cdot 75$ to $1 \cdot 5$, and occasionally 2μ; they were all uniformly stained; a few were curved.

On Bovine Serum.—Growth satisfactory. Morphology, in a 28 days' film, as on horse serum.

On Dog Serum.—In five days a greyish layer of growth was observed. At the end of a fortnight the growth was very copious; and at the end of three weeks there were large, raised, white colonies. In a 14 days' film the bacilli measured from $\cdot 75$ to $1 \cdot 25\mu$, and were straight and uniformly stained.

On Broth.—Small, grey, floating islands were observed at the end of a week. At the end of a fortnight over two-thirds of the surface were covered; the pellicle was thick, and, in places, warty. The surface was completely covered during the fourth week. In a 22 days' film the bacilli measured from 1 to 3μ. A few were curved. Most of the bacilli were uniformly stained, but a few were regularly or imperfectly beaded.

On Glycerin-Agar.—Growth luxuriant. Raised and warty at the end of a fortnight; wrinkled at the end of three weeks; very dense, wrinkled and studded with yellow warts at the end of five weeks. In a 37 days' film the bacilli measured from 1 to 3 and occasionally 4μ; about two-thirds of them were curved; many were notably slender; about one-third were irregularly stained.

On Potato.—Grew readily, forming a fairly abundant growth, with deep yellow pigment in 14 days, and increasing considerably during the following fortnight. In a 37 days' film the bacilli measured from $1 \cdot 5$ to $4 \cdot 5\mu$, averaging about 3μ. The great majority of them were curved and regularly beaded.

SUMMARY.

Growth very good on serum; luxuriant on other media.

VIRUS—H 27. B.D.
(Human Cervical Glands.)

Strain.—G.P. 1216 from ORIGINAL MATERIAL.

Material Received.—Second generation, 12 days old, of culture isolated from G.P. 1216, which was inoculated with original material.

On Horse Serum.—Growth abundant. In a 14 days' film the bacilli were straight and uniformly stained. Their length varied between $1 \cdot 5$ and $\cdot 5\mu$, the average being less than 1μ.

On Bovine Serum.—Growth abundant. When grown on a rich yellow sample of bovine serum, the colonies became deeply pigmented. In a 14 days' film the bacilli were as short as those grown on horse serum; many forms with a slight central constriction were noted.

On Broth.—Growth very vigorous; a warty, wrinkled film covering surface in about three weeks. In a 23 days' film the bacilli were short, slender, homogeneously stained, and from 1 to $2 \cdot 5\mu$ long. The longer forms were curved.

On Glycerin-Agar.—Grew readily, with the production of a copious, very wrinkled growth. In a 29 days' film the bacilli stained uniformly, and were mostly curved. Length from $1 \cdot 5$ to 3μ; average, rather under 2μ.

On Potato.—Rapid, copious, yellow, heaped-up growth. In a 31 days' film the bacilli were long, slender, and curved, often with tapering ends. Some of them stained faintly, but not many were beaded. Extremely few bacilli were either straight or less than 2μ in length. The majority were from $3 \cdot 5$ to 8μ, the average being rather above 4μ.

SUMMARY.

Growth very luxuriant.

VIRUS—H 28. C.L.
(Human Cervical Glands.)

(1.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Third generation, 14 days old, of culture isolated from original material.

On Horse Serum.—The first culture made produced a scanty growth in 10 days; the entire surface of the serum was thinly covered in 18 days; and in 28 days the growth was moderately abundant. Later cultures produced a better, but not a particularly luxuriant, growth. In a 19 days' film the bacilli were uniformly stained and with few exceptions straight. Their length varied from about $1 \cdot 5$ to $\cdot 5\mu$, the average being under 1μ.

On Bovine Serum.—In the first culture made (fourth generation) a few minute colonies were noted at the

margins of the serum in 10 days; in 18 days there were fairly large, discrete, grey colonies scattered over three-fourths of the surface; in 28 days the growth was still of the same type, the colonies being discrete, and no growth being visible on the intervening surface. In the next generation, however, a vigorous growth was produced all over the surface of the serum in six days, and the culture continued to grow abundantly, with the formation of large colonies. In a 14 days' film the only difference noted in the bacilli, compared with those grown on horse serum, was that forms of from 1·5 to 2μ were rather more numerous.

On Dog Serum.—The first culture on this medium yielded a good growth, the type being dull grey and uniform until the fourth week, when white colonies appeared. A second and a third generation were grown on the same medium; they both grew more luxuriantly than the first, and began to produce white colonies and patches at the end of the first week. Morphologically, in 14 days' films, the bacilli from all three cultures were identical. They were almost all straight, all uniformly stained, and rather short, not averaging more than ·75μ.

On Glycerinated Bovine Serum.—At the end of the first week extremely little growth had appeared; on the control tube of the same bovine serum, without glycerin, the surface was well covered, and white, raised colonies were appearing. At the end of the second week the growth was still very poor; it was much better on pure serum. At the end of the third week there was some increase, and many small grey colonies had appeared; the growth on pure serum was better. At the end of the fourth week the growth had increased generally, and the grey colonies were larger and more numerous; the growth on pure serum was still rather better. The growth on glycerinated serum continued to advance slightly, whilst the growth on pure serum remained stationary, and became rather dry. At the end of seven weeks the former showed a scanty dull grey layer, interspersed with many discrete white colonies, the largest of which were the size of a pin-head. In a 21 days' film the bacilli measured from ·75 to 2·5μ, the average being rather less than 1·5μ. All were uniformly stained, and the great majority were straight.

On Broth.—A very delicate, nearly translucent film, speckled with white points, covered the surface in about 5 weeks. In a 34 days' film the bacilli were for the most part straight and uniformly stained, their length varied from 1 to 2·5μ.

On Glycerin-Agar.—A fine grey haze, with some slight, irregular thickening towards the end of the second month. In a 33 days' film the bacilli were all uniformily stained; curved forms were less numerous than straight. The bacilli varied in length from ·75 to 2·5μ.

On Potato.—No growth obtained.

SUMMARY.

Growth fairly good on serum; poor on glycerinated serum; very poor on other media.

(2.)

Strain.—MONKEY 63.

Material Received.—Third generation, eight days old, of culture isolated from Monkey 63.

On Dog and Bovine Serum.—On both these media the first cultures made grew poorly. On dog serum a subculture from the first dog serum tube grew well. On bovine serum the second culture produced a rather scanty growth, but subsequent cultures were much better. On dog serum, in a 16 days' film, the bacilli varied in length from ·75 to 1·5μ, and were straight and uniformly stained. On bovine serum, in a 17 days' film, the bacilli were very similiar to those on dog serum, but a few rather longer forms were noted.

On Broth.—Small, delicate, translucent islands commenced to form towards the end of the third week. The increase was very slow, the surface being not much more than half covered at the end of the sixth week. The pellicle consisted of discrete islands, was speckled, slightly opaque, rather moist, and began to sink after the fifth week. In a 40 days' film the bacilli measured from 1 to 2, and occasionally 2·5μ. They were all uniformly stained, a small number of them were curved.

On Glycerin-Agar.—Growth very poor. No more than a fine grey haze was obtained at the end of two months. In a 70 days' film the bacilli varied in length from ·75 to 1·5μ and occasionally 2μ. They were uniformly stained, and nearly all straight.

On Potato.—No growth was observed before the fifth week. Some light grey streaks were then observed which increased, but only to a very small extent, until the end of the second month. In a 70 days' film the bacilli measured from ·75 to 2μ and occasionally 2·5μ. They were nearly all straight. A few of them were regularly beaded.

SUMMARY.

Growth similar to that of the strain from original material.

VIRUS—H 29. M.F.

(Human Cervical Glands.)

Strain.—G.P. 1329 from ORIGINAL MATERIAL.

Material Received.—Second generation, 12 days old, of culture isolated from G.P. 1329, which was inoculated with original material.

On Horse Serum.—The first culture made was rather poor; subsequent cultures were fairly good. In an 8 days' film the bacilli were uniformly stained and, with few exceptions, straight; their length varied from 2 to ·5μ, and averaged rather under 1μ.

On Bovine Serum.—Growth good. In a 41 days' film the bacilli resembled those grown on horse serum, with the exception that some of them contained globular swellings.

On Dog Serum.—Grew fairly well, preserving the dull grey, uniform type until the third week, when white colonies appeared. In a 14 days' film the bacilli were uniformly stained, mostly straight, and varied in length from ·75 to 1·25 and occasionally 1·5μ, the average being rather less than 1μ.

On Glycerinated Bovine Serum.—At the end of the first week the surface was covered with a thin layer. During the second week this layer became thicker, assumed the appearance of ground glass, and exhibited numerous white specks. There was some further increase during the third and fourth weeks. The ultimate yield was very moist, moderately abundant, and speckled with white colonies. In an 18 days' film the bacilli measured from 2 to 8μ; a large number were from 3 to 5μ long. Most of them were curved; most of them were regularly beaded; there were some globular swellings.

On Broth.—A semi-translucent pellicle, speckled with small white points and patches, covered the surface in about five weeks. In a 25 days' film the bacilli exhibited great irregularity in size and shape, their length varied from 1 to 5μ, forms over 3μ preponderating. Most of the longer forms were curved. Many bacilli were either beaded, or stained irregularly, or showed globular swellings.

On Glycerin-Agar.—Growth slow and scanty, no more than a thin opaque layer with a few thicker points, being formed. In a 33 days' film the bacilli were mostly curved and from 2·5 to 5μ in length. Regularly beaded forms, faintly stained forms, and globular swellings were all numerous.

On Potato.—Doubtful if any increase on the material inoculated.

SUMMARY.

Growth fairly good on serum and glycerinated serum ; poor on other media.

VIRUS—H 30. E.M.

(Human Mesenteric and Bronchial Glands.)

Strain.—G.P. 1338 from ORIGINAL MATERIAL.

Material Received.—Original culture, 35 days old, isolated from G.P. 1338, one of the second series of guinea-pigs inoculated with original material.

On Horse Serum.—Grew well from the commencement. In a 20 days' film most of the bacilli were from ·75 to 1·25μ long, straight, and not beaded; a few rather longer and a few curved forms were also noted.

On Bovine Serum.—Grew well from the commencement. In a 27 days' film the bacilli were identical with those grown on horse serum.

On Dog Serum.—The surface was thickly covered in seven days with a grey layer exhibiting some raised white colonies and patches. In 14 days there was a copious, white, heaped-up growth, which continued to increase during the next fortnight. In a 14 days' film the bacilli were all uniformly stained, with the exception of a few which showed globular swellings ; a moderate number were curved. The average length was about 1·3μ.

On Glycerinated Bovine Serum.—At the end of a week there were many raised yellow colonies, whilst the rest of the surface was uniformly, but rather thinly covered ; a culture of the same age, grown on the same bovine serum, without the addition of glycerin, was identical in character. At the end of a fortnight the whole surface was more thickly covered, and the yellow colonies were larger and more elevated ; the growth on pure serum was identical in character. At the end of three weeks there was considerable further increase ; the growth on pure serum was poorer. At the end of a month there was a very luxuriant, yellow, warty growth ; the growth on pure serum was more uniform and much less copious. In a 19 days' film the bacilli measured from 1·5 to 3·5μ. Most of them were curved. There was a tendency to beading in a large number, but clearly discrete beads were not common. Some bacilli contained globular swellings.

On Broth.—Growth commenced during the second week. At the end of the third week the surface was nearly half covered with opaque, grey, rather warty islands. The pellicle continued to grow vigorously, covering the whole surface, becoming gradually thicker, and showing no tendency to sink. In a 26 days' film all the bacilli were uniformly stained. They measured from 1 to 2·5μ, the average being about 1·5μ ; about half the longer forms were curved.

On Glycerin-Agar.—A thin grey growth, with slightly raised colonies, covered the greater part of the surface in a fortnight. In three weeks the surface was completely covered and definitely wrinkled. In a month's time the growth was moderately thick, raised, and prominently wrinkled. In a 26 days' film the majority of the bacilli were straight, uniformly stained, and measured from ·75 to 1·5μ ; there were also a few curved, not beaded forms, measuring from 2 to 3μ.

On Potato.—In a week's time raised yellow colonies were formed ; there was an abundant increase at the end of a fortnight ; further growth took place during the third and fourth weeks, the ultimate yield being moderately abundant, heaped up, and yellow. In a 26 days' film nearly all the bacilli were curved. Most of them were either regularly beaded or exhibited globular swellings. The length varied from 2 to 7μ ; forms measuring from 4 to 5μ were very frequent.

SUMMARY.

Good on all media, but not quite so good as some viruses on broth, glycerin-agar, and potato.

VIRUS—H 31. L.F.

(Human Cervical Glands.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 19 days old, of culture isolated from original material.

On Horse Serum.—Growth satisfactory. In a 16 days' film the bacilli were from ·75 to 1·5, and occasionally 2μ long, the average being about 1μ. All were uniformly stained, and nearly all were straight.

On Bovine Serum.—Growth satisfactory. Morphology, in a 28 days' film, as on horse serum.

On Dog Serum.—In five days the surface was uniformly covered, and white colonies had begun to appear. The growth had become copious, white, and heaped up at the end of a fortnight. In a 14 days' film the bacilli resembled those grown on horse serum.

On Broth.—At the end of a fortnight two-thirds of the surface were covered with a pellicle which was for the most part delicate and semi-translucent, but exhibited some irregular opaque patches. At the end of three weeks the surface was completely covered and the pellicle was still very delicate. During the fourth and fifth weeks the pellicle became thicker, but not uniformly thicker, and tended to break up. In a 19 days' film the bacilli measured from ·75 to 3μ, occasionally 4μ, the average being about 1·5μ. A considerable number were curved. Most of them were uniformly stained, but there were some globular swellings and a few faintly stained forms. There was no regular beading.

On Glycerin-Agar.—Grew moderately well to begin with, becoming fairly thick and grey at the end of a fortnight. At the end of the third week the growth had assumed a frosted glass appearance. It continued to increase, slowly, until the end of the sixth week. The final growth was of the frosted glass type but irregularly distributed, fairly dense and slightly raised in patches, and with no sign of wrinkling. In a 37 days' film the bacilli measured from 1 to 4μ. They were nearly all either beaded or irregularly stained. More than half were curved.

On Potato.—During the first three weeks a scanty white growth was obtained. The subsequent increase was only slight. In a 37 days' film the bacilli resembled those grown on glycerin-agar.

SUMMARY.

Growth fairly good on serum ; poor on broth ; rather poor on glycerin-agar ; very poor on potato.

VIRUS—H 32. Y.W.

(Human Bronchial Glands and Mesenteric Glands.)

(1.)

Strain.—Rabbit 166 from Original Material.

(Bronchial Gland).

Material Received.—Second generation, 12 days old, of culture isolated from Rabbit 166, which was inoculated with the original bronchial glands.

On Dog and Bovine Serum.—A dog and a bovine serum tube were inoculated from the material received, on which the growth was so scanty as to be hardly visible to the naked eye. These two sub-cultures yielded a thin layer of growth. The next generation grew distinctly better. Later generations grew as well on serum as most of the other viruses. On dog serum, in a 16 days' film, the bacilli varied in length from $\cdot75$ to $1\cdot5\mu$ and were straight and uniformly stained. On bovine serum, in a 28 days' film, forms as short as $\cdot5\mu$ were frequent; the majority of the bacilli varied in length from $\cdot5$ to $1\cdot5\mu$ and were straight and uniformly stained. A few bacilli were from $2\cdot5$ to $3\cdot5\mu$ in length; these were curved and occasionally beaded.

On Broth.—Many floating islands were observed at the end of a fortnight, the layer being about a quarter of an inch in diameter. At the end of a month the greater part of the surface was covered. The pellicle was moderately thick, but of unequal density, and moist; it showed a tendency to curl downwards at the edges. During the fifth week a good deal of the pellicle sank to the bottom, and the surface never became completely covered. In a 33 days' film the bacilli showed marked pleomorphism. About one-third of the total number measured from 1 to 2μ; they were straight, uniformly stained, and rather thick. The remainder of the bacilli were longer, measuring from 2 to $3\cdot5\mu$ and occasionally from 4 to $4\cdot5\mu$; they stained unequally, regular beading being exceptional; globular swellings were very frequent, and many forms were faintly stained; only a small proportion of them were curved.

On Glycerin-Agar.—A grey film, denser in patches, covered the greater part of the surface in a fortnight. This increased slowly, assuming the frosted glass type, but never became copious. In a 56 days' film the bacilli measured from $1\cdot5$ to 6μ. Most of them were curved and not uniformly stained. Regular beading was frequent, and globular swellings were fairly numerous.

On Potato.—Growth scanty and not pigmented. At the end of six weeks there was a thin grey layer over most of the surface. In a 40 days' film the bacilli measured from 1 to 4μ in length and occasionally from 5 to 6μ. The longer forms (3μ or over) were curved and either regularly beaded or showed some tendency to beading or some irregularity of staining. The shorter forms were straight and generally uniformly, but sometimes faintly, stained.

Summary.

Growth fairly good on serum; on broth moist and with a tendency to sink; on glycerin-agar moderately good; on potato poor.

(2.)

Strain.—Original Material (Mesenteric Gland).

Material Received.—Primary culture, 37 days old, isolated from a mesenteric gland.

The first subculture, on dog serum, yielded a copious growth, well covering the surface and showing one or two white, raised colonies in ten days. The second subculture, on the same medium, yielded a fairly copious grey growth in five days.

On Horse Serum.—Growth good. In a 24 days' film the majority of the bacilli were from $\cdot75$ to 1μ long, and were straight and uniform. A moderate number of bacilli were from 2 to 3μ long, about one-half of these longer forms being curved. Occasional globular swellings were noted, and one or two regularly beaded forms.

On Bovine Serum.—Growth good. In a 28 days' film the bacilli measured from $\cdot75$ to $1\cdot5\mu$, rarely 2μ, and averaged not more than 1μ. Very few were curved; all were uniformly stained; forms with a slight central constriction were very common.

On Dog Serum.—Grew rapidly and abundantly, forming a white, heaped-up growth. In a 14 days' film the bacilli measured from $\cdot5$ to $1\cdot5\mu$, and were straight and uniformly stained.

On Broth.—At the end of a week there were several small floating islands. At the end of a fortnight the surface was nearly three-fourths covered with irregular grey islands of varied size and density. At the end of three weeks the surface was completely covered, being thin and irregular on the whole, with some thicker streaks and patches. No further thickening took place. The thicker patches had a tendency to sink. In a 22 days' film the bacilli measured from 1 to $4\cdot5\mu$, the average being over $2\cdot5\mu$. Curved forms were frequent; globular swellings were frequent; some bacilli were regularly beaded.

On Glycerin-Agar.—A fairly uniform, greyish layer was obtained at the end of a fortnight. After this period the increase was slow, steady, and not very copious. At the end of the third week the culture had assumed the frosted glass type; some increase took place up to the end of the sixth week, but the type remained the same, and the total yield was not at all dense. In a 37 days' film the bacilli measured from 1 to 6μ; a large number were from $2\cdot5$ to $4\cdot5\mu$. More than half the bacilli were curved. Only the shortest forms were deeply and uniformly stained. Amongst the rest, faintly stained forms, forms with globular swellings, and irregularly beaded forms, were very frequent.

On Potato.—A slight increase, consisting of minute colonies and greyish white streaks, spreading from the original material, was observed during the first three weeks; but this increase was not maintained, and the total yield was poor. In a 37 days' film the bacilli resembled those grown on glycerin-agar.

Summary.

Growth about equal to the strain from the bronchial gland.

VIRUS—H 33. R.T.

(Human Axillary Gland.)

Strain.—Original Material.

Material Received.—Second generation, 15 days old, of culture obtained from original material.

On Horse Serum.—Growth satisfactory. In a 14 days' film the bacilli were straight, notably uniform, and averaged about ·75μ in length.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli measured from ·75 to 2μ, the average being about 1μ. With the exceptions of a very occasional curved form and one or two which were regularly beaded, the bacilli were straight and uniformly stained.

On Broth.—A pellicle commenced to form before the end of the second week, and covered most of the surface in five weeks. It was very dense and wrinkled. In a 3 days' film the bacilli varied in length from 1·5 to 5μ. The majority of the longer forms were curved and a great many of them were either regularly beaded or exhibited a tendency to beading, the beads in these cases not being quite discrete. A few globular swellings were also noted. Many of the bacilli were notably slender.

On Glycerin-Agar.—At the end of a week the surface was well covered with a uniform greyish growth; this increased, steadily and uniformly, and became wrinkled during the fourth week and somewhat warty. The ultimate yield was a dense and markedly wrinkled layer. In a 36 days' film the bacilli varied in length from 1·5 to 4.5μ. The majority were curved. A great many were either regularly or partially beaded. No other irregularities of staining were present.

On Potato.—Grew readily. An abundant, pigmented, heaped-up growth was obtained in a fortnight. This increased considerably during the following fortnight. In a 19 days' film the bacilli measured from 1·5 to 7μ; a large number were from 3 to 4μ long. Almost all, except the shortest forms, were curved; a great many showed a tendency to regular beading, and thickened, globular swellings were fairly numerous.

Summary.

Growth good on serum, luxuriant on other media.

VIRUS—H 34. C.U.

(Human Cervical Glands.)

Strain.—G.P. 236 from Original Material.

Material Received.—Sixth generation, 93 days old, of culture isolated from G.P. 236, which was inoculated with original material.

On Horse Serum.—The first culture began to grow after a week's delay. Later cultures grew well. In a 20 days' film the bacilli were almost all straight and uniformly stained. Their length varied from 2 to ·5μ, the average being slightly under 1μ.

On Bovine Serum.—Growth good. In a 20 days' film the bacilli resembled those grown on horse serum.

On Dog Serum.—A fairly good but not very luxuriant growth, exhibiting white colonies from the first week onwards. In a 14 days' film the bacilli were rather longer than in serum cultures of most viruses, a large number measuring from 1·5 to 2μ. They were all uniformly stained, and nearly half of them were curved.

On Glycerinated Bovine Serum.—At the end of a week there were several large white colonies which had developed from pieces of original material, but the rest of the surface was poorly covered. The surface was better covered at the end of a fortnight, and some further increase took place during the third and fourth weeks. The total yield was not very good; it consisted of fairly numerous, discrete, greyish-yellow colonies, with not much growth on the intervening surface. In a 21 days' film the bacilli measured from 1 to 3μ, and averaged about 1·5μ. Many of them were curved; a few were beaded, but the great majority were stained uniformly.

On Broth.—The surface was covered in about six weeks with an opaque pellicle of unequal density, many portions of it being thick and others thin. It exhibited no tendency to sink. In a film from a 41 days' culture about half the bacilli were curved. Globular forms were very numerous, and regularly beaded bacilli were sometimes found. Most of the bacilli were from 2 to 3·5μ in length, but there were a few slender, curved bacilli from 4 to 5μ long.

On Glycerin-Agar.—A grey layer was rapidly formed, but the increase was much slower after the first fortnight. The ultimate yield was a dry, moderately dense layer, with a frosted glass appearance on the surface, and without many conspicuous wrinkles or projecting colonies. In a 38 days' film all the bacilli stained deeply and homogeneously; none were beaded. The majority were long and curved (from 2·5 to 4μ). Very few bacilli were as short as 1μ.

On Potato.—At the beginning of the second week the surface was covered with discrete colonies, the largest of which had a yellow tinge. Growth continued until the end of a month, when it was fairly abundant and of a pale yellow colour. In a 31 days' film the bacilli varied in length from about 1 to 6μ. All the longer forms were curved. Regularly beaded forms and forms with globular swellings were numerous.

Summary.

Growth fairly good on serum and glycerinated serum; on other media decidedly good, but the increase not maintained so long as with some other viruses.

VIRUS—H 35. C.B.

(Human Bronchial Gland.)

Strain.—G.P. 571 from Original Material.

Material Received.—Fifth generation, 92 days old, of culture isolated from G. P. 571, which was inoculated with original material.

On Horse Serum.—In spite of the age of the material received the first culture made grew very well, without any initial delay. Subsequent cultures also grew well. In a 27 days' film the bacilli were almost all straight and uniformly stained. Their length varied from 2μ to less than ·75μ, the average being slightly under 1μ. Many forms were found with a slight central constriction.

On Bovine Serum.—Growth as on horse serum. In a 27 days' film the bacilli resembled those grown on horse serum.

On Broth.—The pellicle, which developed readily, was opaque and of unequal thickness, some patches being very dense and others much thinner. It had no tendency

to become moist. In films from a 39 days' growth an occasional regularly beaded form was found; all the rest stained deeply and homogeneously; no globular swellings were noted. Most of the bacilli were short and straight, often with slight central constriction, and measured from 1 to 2μ. Curved forms were not at all numerous; those present measured from 1·5 to 3μ.

On Glycerin-Agar.—In a fortnight there was a good, evenly distributed, grey layer of growth, without any irregular elevations; in a month the growth became leathery and very wrinkled. In a 38 days' film a few bacilli were regularly beaded; the rest all stained deeply and homogeneously. About half the bacilli had a well-marked curve. The majority measured from 2 to 2·5μ; there were some shorter forms, but not many under 1μ.

On Potato.—A moderately abundant, pale yellow, crumb-like growth was formed. In a 31 days' film the bacilli were either uniformly stained or showed only a slight tendency to beading. The majority were from 3 to 6μ in length, though shorter forms also occurred.

SUMMARY.

Growth good, but not as luxuriant as with some viruses.

VIRUS—H 36. M.D.
(Human Mesenteric Glands.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Third generation, 5 days old, of culture isolated from original material.

On Dog and Bovine Serum.—Growth fairly good. On dog serum, in a 13 days' film, the bacilli varied in length from 1 to 2 or 2·5μ, and averaged about 1·5μ. A small number of the bacilli were curved, and a few were beaded. On bovine serum, in a 14 days' film, the bacilli varied in length from ·5 to 1·25μ, and were straight and uniformly stained.

On Broth.—Several minute islands were observed in four days. At the end of a fortnight the increase was only small, but there were several thin, speckled islands. At the end of a month these had increased, but had not covered more than half of the surface. The surface was not completely covered in six weeks; the pellicle remained rather delicate, grey, and partially opaque. In a four days' film, the bacilli varied in length from 1·5 to 3μ. They were uniformly stained; slightly curved forms were fairly numerous. In a 28 days' film the bacilli were indistinguishable from those in the four days' film.

On Glycerin-Agar.—A thin layer, with several small grey colonies, appeared at the end of a week. At the end of a fortnight the growth had assumed the frosted glass type. At the end of a month the growth was very wrinkled but not very dense, and was spreading on to the glass. At the end of six weeks the growth on the agar was denser, and the wrinkles were closely set together; the glass opposite the lower half of the medium was completely covered with growth. In a 22 days' film the bacilli varied in length from 2 (occasionally 1·5) to 4μ; the longer forms were frequently curved; almost all the bacilli were uniformly stained. In a 45 days' film of the same culture the length of the bacilli was about the same, but many were beaded, and many contained prominent globular swellings.

On Potato.—Some slight increase was noted at the end of a fortnight, and there was a grey, speckled, scanty growth at the end of a month. In a 22 days' film the bacilli varied in length from 1·5 to 3μ; the great majority were straight; very few were beaded. In a 45 days' film, from the same culture, the bacilli were identical with those in the 22 days' film.

SUMMARY.

Growth fairly good on serum; slow, and not very dense on broth; good on glycerin-agar, scanty on potato.

VIRUS—H 37. O.J.
(Human Cervical Glands.)

Strain.—G.P. 1408 from ORIGINAL MATERIAL.

Material Received.—A portion of the spleen of G.P. 1408, one of the fourth series inoculated from original material.

The primary culture on bovine serum showed a scanty growth in 15 days; it was then transplanted on to horse serum and covered the surface with a thin layer in 14 days; subsequent cultures were more vigorous.

On Horse Serum.—Growth satisfactory. In a 15 days' film the bacilli measured from ·5 to 1·25μ, the average being about ·75μ; they were all straight and uniformly stained.

On Bovine Serum.—Growth satisfactory. In a 28 days' film the bacilli were slightly longer than on horse serum and were occasionally curved.

On Dog Serum.—A copious white growth was produced in five days. At the end of a fortnight the growth was very abundant, somewhat yellowish in colour, and heaped up. In a 14 days' film the bacilli measured from ·5 to 1·25μ, and were straight and uniformly stained.

On Broth.—A dense, wrinkled pellicle was formed. It commenced to stream off the serum, in a continuous layer, at the end of the first week, and covered the surface in from four to five weeks. In a 22 days' film the bacilli, with the exception of very occasional forms which were as long as 4μ, measured from ·75 to 2·5μ; short forms were very numerous, and the average length was not over 1·5μ. Most of the bacilli were straight and uniformly stained, but there were some with globular swellings.

On Glycerin-Agar.—Grew abundantly, becoming copious, raised, and warty at the end of a fortnight. Vigorous increase took place during the third, fourth and fifth weeks, and the growth became very dense, markedly wrinkled, and studded with yellow warts. In a 37 days' film the bacilli measured from 1 to 4μ, the majority being between 2 and 3μ. About two-thirds of them were curved, and about the same proportion were either regularly beaded or irregularly stained.

On Potato.—A fairly good growth, pigmented deeply yellow, was obtained at the end of a fortnight. During the third, fourth, and fifth weeks the growth increased, becoming abundant and heaped up. In a 37 days' film the bacilli resembled those grown on glycerin-agar, but regularly beaded forms were more frequent.

SUMMARY.

Growth luxuriant.

VIRUS—H 38. J.M.

(Human Cervical Glands.)

Strain.—G.P. 793 from ORIGINAL MATERIAL.

Material Received.—Third generation, 92 days old, of culture isolated from G.P. 793, which was inoculated with original material.

On Horse Serum.—The first culture, owing to the age of the material received, was difficult to start. Later cultures grew well. In a 19 days' film the bacilli were uniformly stained and almost all straight. Their length varied from 1·5 to about ·75μ, the average being less than 1μ.

On Bovine Serum.—Growth as on horse serum. In a 19 days' film the bacilli resembled those grown on horse serum, but a few forms with globular swellings were noted.

On Dog Serum.—Grew well, but preserved the uniform dull grey type until near the fourth week, when white colonies began to appear. In a 14 days' film the bacilli were all straight, uniformly stained, and notably short, averaging not more than ·75μ in length.

On Glycerinated Bovine Serum.—The first week's growth was moderately good, and showed many small white colonies. Subsequent increase was slow, and no further growth occurred after the end of the first month. The ultimate yield was moist, white, distributed in irregular patches, and scanty. In a 21 days' film the bacilli varied in length from ·5 to 3·5μ; a fair number were curved; many were regularly beaded, and many were irregularly stained. The variety, both in size and shape, was notable.

On Broth.—At the end of five weeks the surface was two-thirds covered with a thick pellicle which in some places showed warty elevations and was slightly wrinkled. After this period portions of the pellicle showed a tendency to become moist and fall to the bottom of the flask. In a film from a 39 days' culture the bacilli showed considerable variation. A large number stained irregularly and faintly, and from one-third to one-half were beaded or had globular swellings. Short, straight, deeply stained forms were present in moderate numbers. Of the larger forms the majority were curved. The length of the bacilli ranged from 1 to 3·5μ.

On Glycerin-Agar.—During the second week discrete colonies began to form, the rest of the surface exhibiting hardly any growth. At the end of five weeks these colonies had advanced considerably, but on the rest of the surface the growth was very thin and not even opaque. Very little further increase took place, the ultimate yield consisting of a very thin layer interspersed with some dense patches of irregular outline. In a 35 days' film the bacilli were nearly all curved; a great many were regularly beaded, but some contained globular swellings. The length of the majority varied from 2 to 4μ, but several bacilli were as long as 5μ.

On Potato.—Growth slow and poor; no pigment formed. In a 33 days' film the bacilli were mostly straight and evenly stained (·75 to 1·5μ); some longer, straight forms (2 to 3·5μ), and some forms beaded and irregularly stained were also present.

SUMMARY.

Good on serum; rather poor on glycerinated serum; fairly good on broth; poor on glycerin-agar and potato.

VIRUS—H 39. M.B.

(Human Cervical Glands.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Fourth generation, 30 days old, of culture isolated from original material.

On Dog and Bovine Serum.—Growth good. In a 21 days' film the bacilli grown on dog serum measured from ·5 to 1·5μ and were straight and uniformly stained. The bacilli grown on bovine serum, in a 28 days' film, exhibited the same characters.

On Broth.—Growth commenced at the end of the first week and the surface was covered at the end of the third week. The pellicle was at first of moderate but unequal thickness; it afterwards became thicker, more uniform, and wrinkled. In a 25 days' film the bacilli measured from 1 to 3·5μ. The longer forms were often regularly beaded, and many of them were curved.

On Glycerin-Agar.—The surface was covered with a grey layer in a week, began to be wrinkled at the end of a fortnight, and markedly wrinkled at the end of three weeks. The growth steadily continued, but, though very prominently wrinkled, did not, at the end of the seventh week, exhibit any warty colonies. In a 42 days' film the bacilli measured from 1 to 3·5μ, the longer forms being curved. At least one half of the bacilli were beaded, and some of them exhibited globular swellings. On the whole, the characters of the bacilli were rather irregular.

On Potato.—At the end of a week the surface was covered with small, lemon-yellow colonies. The growth steadily increased until the end of the fifth week, the yield then being fairly abundant and somewhat heaped up. In a 41 days' film the bacilli varied in length from 2 or 3 to about 7μ. Nearly all were curved. A large proportion were regularly beaded, most of the remainder either being irregularly beaded or containing globular swellings.

SUMMARY.

Growth abundant, but not so luxuriant as some viruses.

VIRUS—H 40. J.G.

(Human Testis.)

Strain.—G.P. 1439 from ORIGINAL MATERIAL.

Material Received.—Third generation, 26 days old, of culture isolated from G.P. 1439, which was inoculated with original material.

On Dog and Bovine Serum.—All the cultures made grew well. On dog serum, in a 16 days' film, the bacilli measure from ·5 to 1·5μ; they were all straight and uniformly stained; some were very slender and stained faintly. On bovine serum, in a 28 days' film, the bacilli measured from ·75 to 1·5 or 2μ. They were nearly all straight and almost all uniformly stained; a few bacilli were beaded.

On Broth.—Growth commenced in the first week, and the surface was covered in about five weeks. A thick, tough, warty, and unequal pellicle was formed. In a 21 days' film the bacilli measured from 1 to 3μ. They were frequently curved and nearly all uniformly stained.

On Glycerin-Agar.—The surface was well covered with a grey growth studded with small grey points at the end of a week. Growth continued until the end of the fifth week, the yield being then copious, wrinkled, and warty. In a 30 days' film the bacilli varied in length from $1 \cdot 5$ to 4 or $4 \cdot 5\mu$. Nearly half the bacilli were curved; about one-fourth were beaded, sometimes rather irregularly, but, taking the bacilli on the whole, there was no marked pleomorphism.

On Potato.—At the end of a week the surface was covered with small colonies coloured with a deep yellow pigment. There was a rapid increase during the second week, but the growth was slower afterwards, the total yield being only moderately copious. The pigment was most intense about the third week, being then of a rich brown colour; it became much paler afterwards: In a 37 days' film the bacilli varied in length from 2 to 7μ. They were nearly all curved. About half were beaded; the beading was generally rather irregular.

SUMMARY.

Growth, on the whole, very good.

VIRUS—H 41. A.S.

(Human Knee Joint.)

Strain.—G.P. 1437 from ORIGINAL MATERIAL.

Material Received.—Second generation, 39 days old, of culture isolated from G.P. 1437, which was inoculated with original material.

On Dog and Bovine Serum.—The first cultures made yielded good growths. On dog serum, in a 16 days' film, the bacilli measured from $\cdot 75$ to 2μ, and were straight and uniformly stained. On bovine serum, in a 28 days' film, the bacilli were, on the whole, slightly shorter.

On Broth.—Growth commenced during the first week and at once began to assume the thick, leathery type of pellicle. At the end of the third week nearly the whole of the surface was covered with a pellicle which was for the most part thick and leathery, but exhibited some patches which were thinner than the rest. In a 21 days' film the bacilli measured from 1 to 3 or $3 \cdot 5\mu$. Most of them were straight and uniformly stained; curved or beaded forms, though present, were rare.

On Glycerin-Agar.—An abundant, grey, raised growth was obtained at the end of the first week. At the end of a fortnight the growth was very dense, warty, and beginning to be wrinkled. The ultimate yield was luxuriant, wrinkled, and warty. In a 30 days' film the bacilli measured from 1 to 3μ, the average being not more than $1 \cdot 5\mu$, Many slender and some faintly stained forms were noted. About one-fourth of the bacilli were curved. A few were regularly beaded, and an occasional globular swelling was found.

On Potato.—Growth commenced during the first week and an abundant, raised, lemon-yellow growth was obtained at the end of the third week. In a 37 days' film, the bacilli measured from 1 to $4 \cdot 5\mu$. All except the shortest forms were curved. Most of the bacilli either were regularly beaded or showed globular swellings.

SUMMARY.

Growth luxuriant.

VIRUS—H 42. M.R.

(Human Ankle Joint.)

Strain.—G.P. 1447 from ORIGINAL MATERIAL.

Material Received.—Second generation, 25 days old, of culture isolated from G.P. 1447, which was inoculated from original material.

On Dog and Bovine Serum.—The first cultures made grew well on both of these media. On dog serum, in a 16 days' film, the bacilli measured from $\cdot 5$ to $1 \cdot 5\mu$. They were straight and uniformly stained, but a few faint and slender forms were noted. On bovine serum, in a 28 days' film, the bacilli resembled those grown on dog serum.

On Broth.—The pellicle formed was moderately thick and grew rather slowly, not more than one third of the surface being covered at the end of three weeks; the total surface was covered in about five weeks. In a 20 days' film the bacilli measured from 1 to $3 \cdot 5\mu$. They were frequently curved, nearly all uniformly stained, and only occasionally beaded.

On Glycerin-Agar.—Grew well. Slightly wrinkled at the end of a fortnight. Abundant, very prominently wrinkled, and with some small warty elevations at the end of five weeks. No further increase. In a 30 days' film the bacilli varied in length from 1 or $1 \cdot 5$ to $3 \cdot 5\mu$. More than half were curved. Nearly one half were regularly beaded; some globular swellings were noted.

On Potato.—Grew well for the first fortnight, forming lemon-yellow, confluent, slightly raised colonies. The increase continued, but rather slowly, until the end of the fifth week. In a 37 days' film the bacilli varied in length from 1 to 6μ. Nearly all were curved. Most of them were beaded, more or less regularly. Globular swellings were very numerous and conspicuous.

SUMMARY.

Growth good, but not so vigorous as some viruses.

VIRUS—H 43. F.F.

(Human Knee Joint.)

Strain.—G.P. 1425 from ORIGINAL MATERIAL.

Material Received.—Third generation, eight days old, of culture isolated from G.P. 1425, which was inoculated with original material.

On Dog and Bovine Serum.—Growth good. On dog serum, in a 9 days' film, the bacilli were notably small and uniform in length, the average being rather under ·75μ. They were all straight and uniformly stained. On bovine serum, in a 15 days' film, the bacilli were identical with those found on dog serum.

On Broth.—The broth was half covered with a dense, tough pellicle at the end of a fortnight. At the end of three weeks it was completely covered, and at the end of a month the pellicle was particularly dense and markedly wrinkled. In a 33 days' film the bacilli varied in length from ·75 to 3μ, and averaged about 1·5μ. They were uniformly stained with the exception of an occasional globular swelling. About one-third were curved.

On Glycerin-Agar.—The growth was exceptionally rapid and luxuriant. It was abundant in five days, raised and warty in a fortnight, and formed a very thick layer studded with numerous warty elevations at the end of a month. In a 20 days' film the bacilli exhibited much variety. Many were from 1 to 2μ in length, straight and uniformly stained. Many were from 2 to 3·5μ long; these were curved and about half of them were beaded. A few were from 4 to 5μ long and were curved and beaded.

On Potato.—Many lemon-yellow colonies appeared in five days. The growth increased rapidly until the end of the fifth week, forming a heaped-up, deep yellow growth of the pulled-bread type. In a 20 days' film the bacilli varied in length from 1 to 7μ. About two thirds of the bacilli were short, straight, and uniformly stained. The remaining third were notably long; they were curved, generally uniformly stained, and only occasionally beaded.

SUMMARY.

Very luxuriant.

VIRUS—H 44. D.C.

(Human Cervical Glands.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 34 days old, of culture isolated from original material.

On Dog and Bovine Serum.—The first cultures grew well on both these media. On dog serum, in a 16 days' film, the bacilli varied in length from ·75 (occasionally ·5) to 1·5μ, and were all straight and uniformly stained. On bovine serum, in a 21 days' film, a few bacilli were longer, an occasional bacillus was beaded, and there were many slender and faintly stained forms.

On Broth.—A pellicle, dense but not uniformly dense, was formed which covered two-thirds of the surface in three weeks and the whole of the surface in five weeks. In a 21 days' film the bacilli varied in length from 1 to 3μ; they were frequently curved, but rarely beaded.

On Glycerin-Agar.—Surface covered with an opaque, grey, slightly raised growth in a week. The growth was much denser, and exhibited some small nodular points at the end of the second week. During the third week the growth increased in thickness; during the fourth week it became wrinkled; and at the end of the fifth week warty projections were noted. In a 30 days' film the bacilli measured from 1 to 4μ. About half the bacilli were curved; about a quarter were beaded, and a few were stained irregularly.

On Potato.—Very pale, yellow colonies appeared at the end of the first week, and then increased steadily, but not very rapidly. At the end of five weeks the surface was thickly covered, slightly heaped up, and pale yellow. In a 37 days' film the bacilli measured from 1·5 to 5μ. Most of them were curved, and either were regularly beaded or exhibited globular swellings.

SUMMARY.

Growth good on all media.

VIRUS—H 45. F.M.

(Human Bronchial Gland.)

Strain.—G.P. 1137 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 10 days old, of culture isolated from G.P. 1137, which was inoculated with original material.

On Horse Serum.—A good growth was formed in 4 days and the culture continued to grow vigorously during the whole of the first month. Subsequent cultures always yielded very good growths. In a 15 days' film the bacilli were all uniformly stained and, with few exceptions, straight. Their length varied from 3 to ·5μ, the average being slightly over 1μ.

On Bovine Serum.—Growth identical with that on horse serum. In a film from a 20 days' old culture a few beaded and several faintly or irregularly stained forms were noted.

On Dog Serum.—Raised white colonies appeared during the first week, and the ultimate yield was very copious, white, and warty. In a 15 days' film the bacilli measured from ·75 to 2·5μ, the average being rather less than 1·5μ. Several of the longer forms were curved. All the bacilli were uniformly stained.

On Glycerinated Bovine Serum.—At the end of the first week there was an abundant, uniform growth, amongst which many white points were scattered. There was a rapid increase during the second week, and the growth became yellower. Further increase took place during the third, fourth, and fifth weeks. The ultimate yield was a dense yellowish layer, the surface of which showed many projecting yellow colonies, and was otherwise of a somewhat frosted appearance. In a 21 days' film the bacilli measured from 1 to 3μ; they were uniformly stained; more than half of them were curved.

On Broth.—Grew with remarkable rapidity, covering the surface in 12 days with a thick, opaque, fairly homogeneous pellicle. This subsequently became denser, warty, and wrinkled. In a preparation from an 8 days' growth, the bacilli measured from 1·5 to 3·5μ, were more frequently straight that curved, and frequently contained globular swellings.

On Glycerin-Agar.—Grew very vigorously, forming a wrinkled layer, with warty elevations, in 16 days. This became very dense in less than a month. In a 19 days' film the bacilli stained uniformly, measured from 1 to 3μ, and were more frequently straight than curved.

On Potato.—Grew with exceptional rapidity, producing a copious, crumb-like, brownish-yellow layer in 14 days. In a 19 days' film the majority of the bacilli were curved, beaded, or irregularly stained, and from 2 to 4·5μ long.

SUMMARY.

Very luxuriant.

VIRUS—H 46. H.W.

(Human Knee Joint.)

Strain.—G. P. 1444 from ORIGINAL MATERIAL.

Material Received.—Third generation, 8 days old, of culture isolated from G.P. 1444, which was inoculated with original material.

On Dog and Bovine Serum.—Grew abundantly. On dog serum, in a 16 days' film, the bacilli measured from ·75 to 1·5μ, occasionally 2μ. They were uniformly stained and rarely curved. On bovine serum, in a 17 days' film, the characters of the bacilli were identical with those found on dog serum.

On Broth.—The surface was half covered in a fortnight, and completely covered in a month. The pellicle was very dense and tough. In a 33 days' film the bacilli varied in length from 1 to 3·5μ. About one-third of them were curved. All were uniformly stained.

On Glycerin-Agar.—Grew with great luxuriance, forming a copious, raised, white, and warty layer in a fortnight, and becoming particularly dense, for an agar culture, at the end of a month. In a 20 days' film the bacilli varied in length from 2 to 3·5μ. About one-half were curved and one-quarter were either regularly beaded or exhibited globular swellings.

On Potato.—Many yellow colonies were visible in five days. The growth was abundant, heaped up, and pale yellow at the end of a fortnight. Growth continued vigorously, and in the sixth week the culture was heaped up, of the pulled-bread type, and brownish-yellow in colour. In a 20 days' film the bacilli varied in length from 1·5 to 6μ. All, except the short forms, were curved and beaded; globular thickenings were fairly numerous.

SUMMARY.

Very luxuriant.

VIRUS—H 47. S.B.

(Human Hip Joint.)

Strain.—G.P. 1430 from ORIGINAL MATERIAL.

Material Received.—Third generation, 8 days old, of culture isolated from G.P. 1,430, which was inoculated from original material.

On Dog and Bovine Serum.—Growth luxuriant. On dog serum, in a 16 days' film the bacilli varied in length from ·5 or ·75 to 1·25 or 1·5μ. They were straight, uniformly stained, and rather slender. On bovine serum, in a 17 days' film, the characters of the bacilli were almost identical with those on dog serum; possibly they were slightly longer.

On Broth.—The surface was about a quarter covered at the end of a fortnight and nearly covered at the end of a month. The pellicle was moderately thick, speckled with warty points, and rather broken up into islands. It did not become moist or exhibit any tendency to sink. In a 33 days' film the bacilli varied in length from 1 to 3·5μ, with occasional forms as long as 4 or 5μ. Nearly one half the bacilli were curved. The bacilli were uniformly stained, with the exception of an occasional irregularly beaded form.

On Glycerin-Agar.—Growth luxuriant. Dense and warty in a fortnight and very copious and warty in a month. In a 20 days' film the bacilli varied in length from 2 to 4·5μ. Two-thirds of them were curved. Over one-third were either regularly beaded or exhibited globular swellings.

On Potato.—Many lemon-yellow colonies appeared in five days and the growth increased fairly rapidly until the end of the first month, consisting at that time of a raised mass of pale yellow colonies. In a 20 days' film the bacilli varied in length from 1·5 to 3·5 or 4μ. Short and straight forms were much more frequent than long forms. Not more than one-tenth of the bacilli were beaded. Some of the bacilli were not acid-fast.

SUMMARY.

Growth luxuriant.

VIRUS—H 48. W.P.

(Human Lung.)

Strain.—G.P. 1493 from ORIGINAL MATERIAL.

Material Received.—Second generation, 22 days old, of culture isolated from G.P. 1493, which was inoculated with original material.

On Bovine Serum.—Growth abundant. In a 16 days' film the majority of the bacilli varied in length from ·75 to 2μ and were straight and uniformly stained. There were also some longer forms, from 2·5 to 3·5μ. These were often curved and occasionally beaded; some contained deeply stained thickenings.

On Broth.—Growth commenced at the end of the first week and the surface was covered at the end of a month. The pellicle was thick and showed no tendency to sink. In a 21 days' film the bacilli varied in length from 1 to 3μ and were uniformly stained. Nearly half of them were curved.

On Glycerin-Agar.—A copious growth was produced which became prominently wrinkled in a fortnight, the ultimate yield being thick, wrinkled, and warty. In a 27 days' film the bacilli varied in length from 1·5 to 4·5μ and were nearly all curved. The staining was very irregular; globular swellings were numerous, and beading was common.

On Potato.—An abundant, heaped up, deep yellow growth was produced in a fortnight and in five weeks the growth had assumed the typical pulled-bread appearance. In a 40 days' film the bacilli varied in length from 1 to 8μ; with the exception of some short, thick straight forms from 1 to 1·5μ, the bacilli were almost all curved and beaded. Some contained irregular deeply stained thickenings.

SUMMARY.

Growth luxuriant.

VIRUS—H 49. T.C.

(Human Mesenteric Gland.)

(1.)

Strain.—Original Material.

Material Received.—Second generation, 12 days old, of culture isolated from original material.

On Bovine Serum.—Growth abundant. In a 21 days' film the bacilli were for the most part from ·75 to 1·5μ in length and were straight and uniformly stained. A few were from 2 to 2·5μ long, uniformly stained and curved.

On Broth.—Growth commenced at the end of the second week; it proceeded very slowly and did not succeed in covering more than two-thirds of the surface. The pellicle was rather thin and somewhat opaque. In a 27 days' film the bacilli varied in length from 1 to 3μ; they were uniformly stained and occasionally curved.

On Glycerin-Agar.—Increase took place very slowly, the yield at the end of eight weeks being a moist, grey, scanty growth containing some groups of denser points and nodules. In a 27 days' film the great majority of the bacilli varied in length from 1 to 3μ; a few measured from 4 to 4·5μ. Many of the longer forms were curved. Most of the bacilli were uniformly stained, but some contained globular swellings and some were beaded.

On Potato.—A scanty, non-pigmented growth developed during the second and third weeks but made no subsequent increase. In a 45 days' film the bacilli varied in length from 1 to 4μ, the majority being not over 2μ. Most of them were straight and uniformly stained, but some curved forms and some partially beaded forms occurred.

Summary.

Growth good on serum; poor on broth; poor and moist on glycerin-agar; poor on potato.

(2).

Strain.—Calf 787.

Material Received.—Third generation, 20 days old, of culture isolated from Calf 787.

On Bovine Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ. They were uniformly stained. A few were curved.

On Broth.—At the end of three weeks the surface was covered with a thin greyish layer, which shortly afterwards became moist and sank to the bottom of the flask. In a 22 days' film the bacilli varied in length from 1 to 3·5 or occasionally 4·5μ. The longer forms were curved. The staining was very irregular.

On Glycerin-Agar.—Growth was fairly good during the first month, a grey layer of unequal density but with some thick patches being produced. No further increase took place. In a 42 days' film the bacilli varied in length from 1 to 3·5 or occasionally 4.5μ. The longer forms were curved. The staining was very irregular.

On Potato.—The surface was covered in a month with a rather thin, grey layer, exhibiting several raised colonies. No further increase took place. In a 60 days' film the bacilli varied in length from ·75 to 3μ. They were straight and, on the whole, uniformly stained, but some irregularly stained forms were noted.

Summary.

Growth poor on broth, fairly good on glycerin-agar, rather poor on potato. On the whole, rather better than the strain of this virus isolated from the original material.

(3).

Strain.—Calf 797.

Material Received.—Fourth generation, 20 days old, of culture isolated from Calf 797.

On Bovine Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2μ. A few were curved.

On Broth.—The surface was covered in about three weeks with a thin, opaque, uniform layer, which soon afterwards became moist and sank to the bottom of the flask. In a 22 days' film the bacilli varied in length from 1 to 3 μ. They were nearly all straight and uniformly stained.

On Glycerin-Agar.—A thin, opaque layer with some denser patches was formed in three weeks. Very little increase took place subsequently. In a 42 days' film the bacilli varied in length from ·75 to 3·5 μ, the longer forms being curved. There was some tendency to beading; some of the bacilli had thickened ends; globular swellings were frequent.

On Potato.—A thin, grey layer was formed in three weeks. At the end of two months minute, crumb-like grey colonies were seen all over the surface. In a 60 days' film the bacilli varied in length from about ·75 to 4 μ. The staining was very irregular.

Summary.

Growth rather inferior to the strain from Calf 787; about equal to the strain from original material.

VIRUS—H 50. P.H.

(Human Lung.)

Strain.—G.P. 1490 from Original Material.

Material Received.—Second generation, 21 days old, of culture isolated from G.P. 1490.

On Bovine Serum.—Growth good. In a 22 days' film the bacilli, for the most part, varied in length from ·75 to 2μ, averaged rather less than 1·5μ, and were straight and uniformly stained. A small number measured from 2·5 to 3μ; these were curved and occasionally beaded. Some rather slender forms were noted. On the whole, the bacilli were rather irregular for a serum culture.

On Broth.—Growth commenced during the second week and the surface was covered at the end of five weeks. The pellicle was thick for the most part, but

not uniformly thick. In a 27 days' film the bacilli varied in length from 1 to 3μ. Many were curved and regularly beaded; many contained globular swellings, and forms with tapering ends were frequent.

On Glycerin-Agar.—Growth was abundant but not very rapid, a dense, almost uniform, layer being obtained in five weeks. In a 27 days' film the bacilli varied in length from 1 or 1·5 to about 4μ. They were frequently curved and generally uniformly stained.

On Potato.—A deep yellow, crumb-like, fairly abundant growth was produced. In a 40 days' film the bacilli varied in length from 1·5 to 6 or 7μ, the majority being over 4μ. There were however a few short, thick, straight forms. With these exceptions the bacilli were nearly all curved and regularly beaded.

SUMMARY.

Growth decidedly good, but less luxuriant than with some viruses.

VIRUS—H 51. H.M.

(Human Lung.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Second generation, 13 days old, of culture isolated from original material.

On Bovine Serum.—Growth good. In a 23 days' film the bacilli varied in length from ·75 to 1·5 μ, with occasional forms between 2 and 3 μ. They were all straight, or only slightly curved, and uniformly stained. Forms with a slight central constriction were rather frequent.

On Broth.—Growth commenced at the end of the second week and the surface was covered at the end of the fifth week. The pellicle was thick and showed no tendency to sink. In a 52 days' film about half the bacilli were about 1 μ in length, straight, and uniformly stained. The rest were from 2 to 3·5 μ long, generally stained uniformly, and often curved.

On Glycerin-Agar.—Growth rapid and abundant, becoming wrinkled and warty in three weeks and continuing to increase in density until the end of the sixth week. In a 41 days' film, most of the bacilli varied in length from ·75 to 4 μ; some of them measured from 5 to 6 μ. Most of the bacilli were curved; the longer were either regularly beaded or irregularly stained.

On Potato.—Growth abundant, fairly rapid, and coloured with a deep brown pigment. The brown colonies became prominent in the second week and then steadily extended until the end of the sixth week. In a thirty days' film the bacilli varied in length from ·75 to 4·5 μ. The longer were curved and with a tendency to beading. Short, straight, and even coccal forms were fairly numerous.

SUMMARY.

Growth abundant.

VIRUS—H 52. T.F.

(Human Lung.)

Strain.—G.P. 1567 from ORIGINAL MATERIAL.

Material Received.—G. P. 1567. Killed 14 days after inoculation. Cultures were made from the spleen, where the bacilli were moderately numerous. The bacilli varied in length from 2 to 6 μ, the average being between 3 and 4 μ. About half the bacilli were curved; a few were regularly beaded and many contained globular swellings.

On Bovine Serum.—Colonies were first definitely visible in 8 days. At the end of a fortnight the surface was well covered with minute semi-translucent colonies which were closely set together, and gave the surface a fine, ground-glass appearance. At the end of a month all the tubes (4 were inoculated) were covered with a moderately dense, opaque layer, amongst which were several raised white colonies. In a 24 days' film, made from a primary culture, the bacilli varied in length from ·75 to 2 or 2·5 μ. Nearly all were straight and uniformly stained. A few were slightly curved and an occasional beaded bacillus was found. Forms with a central constriction were common.

On Glycerinated Bovine Serum.—Three tubes were inoculated with an emulsion from the spleen. In one, very minute colonies were just visible about the eleventh or twelfth day; growth was then rapid, and at the end of a fortnight was more abundant than on pure serum, and whiter; at the end of a month there was a dense, continuous layer of growth all over the surface, with large, raised, white colonies. In the two other tubes inoculated, no colonies were visible until between the second and the third weeks. At the end of a month, in one of these tubes the surface was covered with small white colonies which were nearly coalescent over most of the surface, and in some patches were heaped up; the other tube, at the same date, exhibited several large (up to 2 mm.), discrete colonies, the rest of the surface being bare. In a 24 days' film, from a primary culture, the bacilli varied in length from 1 to 4·5 μ. Most of the longer bacilli were curved. More than half were either regularly beaded or irregularly stained. Many bacilli contained deeply stained central thickenings which were not globular.

Subcultures on Bovine and Horse Serum.—It was observed that two or three generations grew very poorly. This, however, was only a temporary phase; subsequent cultures were found to grow as well as the majority of other viruses on these media.

On Broth.—Growth commenced at the beginning of the second week, and a grey, fairly thick, and warty pellicle was produced. In one flask growth ceased at the end of a month, when the surface was half covered; but in a second flask the surface became completely covered in the fifth week and began to spread up the glass. In a 50 days' film the bacilli varied in length from 1·5 to 3·5 μ. Most of them were curved, and nearly half were beaded.

On Glycerin-Agar.—Growth commenced rapidly, the surface being well covered with a continuous, grey, very wrinkled layer in three weeks. Very little further increase took place, and at the end of eight weeks the growth had not the dense, warty appearance which is produced by more luxuriantly growing viruses. In a 39 days' film the bacilli varied in length from 1 to 5 μ, the majority being between 3 and 4 μ. All but the shortest were curved and beaded; some exhibited globular swellings.

On Potato.—The first three tubes yielded hardly any growth; in the fourth a definite, scanty, pale yellow growth was slowly produced. In a 35 days' film the bacilli varied in length from 1·5 to 4·5μ. They were, for the most part, curved and uniformly stained.

SUMMARY.

Growth good on the whole, but somewhat uncertain.

VIRUS—H 53. D.H.

(Human Lupus.)

(1.)

Strain.—G.P. 1482 from Original Material.

Material Received.—Second generation, 21 days old, of culture isolated from G.P. 1482, which was inoculated with original material.

On Bovine Serum.—Growth good. In a 22 days' film the bacilli varied in length from ·75 to 1·5 or 2μ and were straight and uniformly stained.

On Broth.—Growth commenced in the second week and continued until the end of the third week, when about two-thirds of the surface were covered. The pellicle then became moist and began to sink. The pellicle was of unequal density, being almost parchment-like in some places and as thin as tissue paper in others. In a 27 days' film the bacilli, with the exception of some coccal forms, measured from 1 to 3μ, and, except the longest, were straight. The staining was very irregular and there were enormous numbers of thickened, spore-like bodies.

On Glycerin-Agar.—The increase was slow and the growth was of the frosted glass type. At the end of eight weeks there was a somewhat scanty grey layer with some denser points and patches. In a 27 days' film the bacilli varied in length from 2 to 4μ. Most of them were curved and stained irregularly; some were regularly beaded.

On Potato.—A scanty increase took place during the first month, exhibiting faint yellow pigmentation. In a 40 days' film there were many short forms (·75 to 1·25μ) and some longer (2·5 to 3·5μ). Most of the bacilli were straight; the longer forms were often beaded.

Summary.

Growth good on serum; on broth uncertain and with a marked tendency to sink; on glycerin-agar and potato rather scanty.

(2).

Strain.—Calf 905.

Material Received.—Second generation, 9 days old, of culture isolated from Calf 905.

On Horse Serum.—Growth good. In a 12 days' film the bacilli varied in length from ·75 to 2·5μ, the average being slightly over 1μ. They were straight and uniformly stained.

On Broth.—A moderately thick, uniform pellicle was rapidly formed and covered most of the surface in about three weeks; it then became moist and sank to the bottom of the flask. In a 21 days' film the bacilli varied in length from 1 to 4μ. The majority were curved, beading was frequent, and irregularly stained forms were also present.

On Glycerin-Agar.—A thin grey layer was formed at the end of the first week. This steadily increased and became somewhat raised and granular during the second and third weeks. Further increase took place during the fourth and fifth weeks, the ultimate yield being fairly abundant, granular, and slightly wrinkled. In a 44 days' film the bacilli varied in length from ·75 to 3·5μ. The majority were straight. Many of the longer forms were stained irregularly.

On Potato.—A moderate amount of growth was obtained, with the formation of raised, grey, crumb-like colonies. No pigment was formed. In a 56 days' film the bacilli varied in length from 1 to 4·5 or 5μ, the longer forms being curved. Staining was very irregular.

Summary.

Growth rather better than the strain of original material through G.P. 1482.

VIRUS—H 54. C.W.

(Human Bronchial Gland.)

Strain.—G.P. 1609, from Original Material.

Material Received.—Fourth generation, 15 days old, of culture isolated from G.P. 1609, which had been inoculated with original material.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli varied in length from ·75 to 2 μ, and were uniformly stained. A few were curved.

On Broth.—A thick pellicle was formed which nearly covered the surface in a month, and showed no tendency to sink. In a 22 days' film the bacilli varied in length from 1 to 4 or 4·5 μ. The majority were curved. A few were beaded or irregularly stained.

On Glycerin-Agar.—A luxuriant, wrinkled and warty growth was obtained in a fortnight. The total yield was very abundant. In a 42 days' film the bacilli varied in length from ·75 to 3·5 or 4 μ. The majority were curved and either regularly beaded or irregularly stained.

On Potato.—Growth fairly good, and deep yellow in colour. In a 42 days' film the bacilli varied in length from 1·5 to 4·5 μ. They were slender, mostly curved, and often beaded or irregularly stained.

Summary.

Growth luxuriant.

VIRUS—H 55. R.D.

(1.)

(Human Bronchial Gland.)

Strain.—Original Material.

Material Received.—Third generation, 15 days old, of culture isolated from the original bronchial gland.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli varied in length from ·5 to 1·5 μ. A few were curved and irregularly stained.

On Broth.—A pellicle which was thick but of unequal density nearly covered the surface in a month. At the end of the fifth week the surface was completely covered and the pellicle was growing up the glass. In a 22 days' film the bacilli resembled those grown on serum. In a 49 days' film of the same culture, rather longer forms (2 to 3μ) were also noted; these were often curved and sometimes beaded.

On Glycerin-Agar.—An abundant, wrinkled growth was formed during the first fortnight. The ultimate yield was thick, very wrinkled, and slightly warty. In a 38 days' film the bacilli varied in length from ·75 to 4 μ. Short forms were very numerous. About half were curved. Some bacilli were stained irregularly, but the majority were stained uniformly.

On Potato.—A yellow layer of growth appeared during the second week. The total yield, at the end of two months, was moderately thick and somewhat heaped up. In a 38 days' film the bacilli varied in length from 1 to 3·5 or 4μ short; straight forms were very numerous. The longer forms were often curved. A few were regularly beaded. The staining, on the whole, was very uniform.

Summary.

Growth luxuriant.

(2.)

(Human Mesenteric Gland.)

Strain.—Original Material.

Material Received.—Third generation, 15 days old, of culture isolated from the original mesenteric gland.

On Bovine Serum.—Growth good. In a 15 days' film the bacilli varied in length from ·5 to 1·5μ. A few bacilli were curved and irregularly stained.

On Broth.—A thick pellicle was obtained which covered the surface in about a month. In a 22 days' film the bacilli varied in length from 1 to 2·5μ, and in general appearance resembled those grown on serum.

On Glycerin-Agar.—An opaque layer, of the ground glass type, was formed in a fortnight. This gradually became thicker, granular, and finely wrinkled, and spread on to the glass. In a 42 days' film the bacilli varied in length from ·75 to about 4μ. About half were curved. Most were stained uniformly, but some of the longest were regularly beaded.

On Potato.—A rather scanty growth was obtained. It was grey during the first three weeks, but became yellow and crumb-like afterwards. In a 42 days' film the bacilli resembled those grown on serum.

Summary.

Growth fairly good, but not so luxuriant as the culture obtained from the bronchial gland.

VIRUS—H 56. F.T.

(Human Lung.)

Strain.—G.P. 1682 from Original Material.

Material Received.—Fifth generation, 18 days old, of culture isolated from G.P. 1682, which was inoculated with original material.

On Horse Serum.—Growth good. In a 14 days' film the majority of the bacilli varied in length from ·75 to 2μ. A few measured from 2·5 to 3μ. They were all uniformly stained. The longer forms were curved.

On Broth.—The surface was covered with a thick pellicle in about a month. In a 22 days' film the bacilli varied in length from 1 to 3·5μ. A moderate number were curved. The majority were uniformly stained but some were beaded and a few exhibited globular swellings. A branched form was found.

On Glycerin-Agar.—A thick, wrinkled, and warty growth was obtained in three weeks. In a 42 days' film the bacilli varied in length from 1 or 1.5 to 6μ; curved, regularly beaded forms were very common. No branched forms were found.

On Potato.—The surface was covered with a thin, brown layer in a fortnight. Some slight increase took place during the three following weeks. In a 56 days' film the bacilli varied in length from 1 to 4·5μ. The majority were curved. Regular beading was less common than irregularities of staining.

Summary

Growth luxuriant.

VIRUS—H 57. B.J.

(1).

(Human Mesenteric Gland.)

Strain.—G.P. 1802 from Human Mesenteric Gland.

Material Received.—Fourth generation, 10 days old, of culture isolated from G.P. 1802, which had been inoculated with the human mesenteric glands.

On Bovine and Horse Serum.—Growth good. In a 14 days' film, from bovine serum, the bacilli varied in length from ·75 to 2μ and averaged about 1μ. They were straight and uniformly stained.

On Broth.—The cultures grew slowly, with the formation of a moderately thick but unequal pellicle, covering most of the surface in six weeks. The pellicle showed no tendency to become moist or sink. In a 28 days' film the bacilli varied in length from 1 to 3μ. About half were slightly curved. The staining was uniform on the whole.

On Glycerin-Agar.—A thick, very wrinkled growth was produced in a fortnight. It subsequently exhibited many small warty elevations. In a 56 days' film the bacilli varied in length from 1 to 8μ. Most of them were curved. Regular beading was very common. Two branched forms were noted.

On Potato.—A fairly thick, crumb-like yellow growth was obtained. In a 56 days' film the bacilli, for the most part, varied in length from 1 to 3μ. Longer forms (4 to 5μ) were also found. About half the bacilli were curved. The staining was notably uniform.

Summary.

Growth abundant on the whole, but pellicle on broth not very dense.

(2).

(Human Liver.)

Strain.—G.P. 1807 from Human Liver.

Material Received.—Second generation, 13 days old, of culture isolated from G.P. 1807, wihch had been inoculated with the human liver.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 1·5μ (rarely 2 to 2·5μ) and were straight and uniformly stained.

On Broth.—Growth was slow and of moderate but unequal density. There was no tendency to sink. The surface became covered in six weeks. In a 28 days' film the bacilli varied in length from 1 to 4·5μ. Curved and regularly beaded forms were numerous.

On Glycerin-Agar.—The growth was rapid, dense, and very prominently wrinkled. In a 42 days' film the bacilli varied in length from 1·5 to 5μ. Most of them were curved. Beading was frequent, and some irregular thickenings were noted. A few branched forms were found.

On Potato.—A fairly abundant, brownish yellow growth was obtained. In a 56 days' film the bacilli varied in length from 1 to 3 . Curved and beaded or irregularly stained forms were rare.

Summary.

Growth luxuriant on glycerin-agar, but rather slow on broth.

(3).

(Human Lung.)

Strain.—G.P. 1800 from Human Lung.

Material Received.—Third generation, 9 days old, of culture isolated from G.P. 1800, which had been inoculated with the human lung.

On Horse Serum.—Growth good. In a 12 days' film the bacilli varied in length from ·75 to 2·5μ and were straight and uniformly stained.

On Broth.—In one flask an opaque, fairly thick pellicle, of unequal density and with no tendency to sink, covered most of the surface in three weeks. In another flask a very thick, wrinkled pellicle was formed. In a 22 days' film the bacilli varied in length from ·75 to 3μ. The majority were straight and uniformly stained.

On Glycerin-Agar.—A dense, warty, and wrinkled growth was readily obtained. In a 50 days' film the bacill varied in length from 1 to 4μ; the majority were curved and uniformly stained, or with a slight tendency to beading.

On Potato.—A fairly abundant growth, exhibiting a brown pigment, was readily obtained. In a 56 days' film the bacilli varied in length from ·5 to 4·5μ. Most of them were curved. Beading was common and there were also many forms which stained irregularly and exhibited globular thickenings.

Summary.

Growth luxuriant.

VIRUS—H 58. F.G.

(1).

(Human Cervical Gland.)

Strain.—G.P. 1816 from Human Cervical Gland.

Material Received.—Fourth generation, 9 days old, of culture isolated from G.P. 1816, which had been inoculated with the human cervical gland.

On Horse and Bovine Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 2·5μ and were nearly all straight and uniformly stained.

On Broth.—A thick pellicle was readily formed and covered the surface in a month; it showed no tendency to sink. In a 27 days' film the bacilli varied in length from about 1 to about 4μ; a large proportion of the bacilli were curved and either beaded or stained irregularly.

On Glycerin-Agar.—The surface became slowly covered with a grey growth which exhibited some denser patches, but never became copious. In a 48 days' film the bacilli varied in length from 1 to 3·5μ. More than half were curved; irregularly stained forms were moderately numerous.

On Potato.—A fairly abundant, yellowish growth was formed. In a 58 days' film the bacilli varied in length from 1 or 1·5 to 5μ (occasionally longer). Nearly all the bacilli were curved. The majority were regularly beaded. Globular thickenings were fairly numerous.

Summary.

Growth poor on glycerin-agar, but fairly abundant on other media.

(2).

(Human Lung.)

Strain.—G.P. 1812 from Human Lung.

Material Received.—Second generation, 9 days old, of culture isolated from G.P. 1812, which had been inoculated with the human lung.

On Bovine and Horse Serum.—Growth good. In a 12 days' film, from bovine serum, the bacilli varied in length from 1 to 2·5 or occasionally 3μ. With the exception of a few curved and a few irregularly stained forms, they were straight and uniformly stained.

On Broth.—An opaque, rather thin pellicle slowly formed. It showed no tendency to sink but gradually formed areas of denser material. The surface was covered in about 5 weeks. In a 28 days' film the bacilli varied in length from 1 to 3·5μ. Most of them were curved. Many stained faintly and irregularly.

On Glycerin-Agar.—Growth commenced as discrete grey colonies closely set together all over the surface. At the end of the third week the growth formed a uniform grey, granular, wrinkled pellicle over the lower half of the tube, and subsequently the upper part of the growth gradually increased in density. In a 50 days' film the bacilli varied in length from 1 to 3·5μ. They were uniform on the whole, but showed some tendency to beading. About one-third were curved.

On Potato.—A scanty, grey, crumb-like growth was slowly formed. In a 56 days' film the bacilli varied in length from 1 to 3μ. They were uniformly stained and nearly all were straight.

Summary.

Growth fairly abundant, but rather slow. No pigment noted on potato. Distinctly better than the previous strain on glycerin-agar.

VIRUS—H 59. L.B.

(1).

(Human Mesenteric Gland.)

Strain.—G.P. 1850 from Human Mesenteric Gland.

Material Received.—G.P. 1850.

Primary Cultures.—Cultures were made, from the spleen and a sternal gland, on egg, bovine serum, and glycerinated bovine serum. On the egg medium the surface became well covered with minute points of growth in about three weeks. On the bovine serum no visible growth was obtained. On the glycerinated bovine serum several discrete white colonies were formed in about three weeks. The strain was continued from the egg culture.

On Horse Serum.—Growth in subculture fairly satisfactory, not so poor as with the other strains of this virus. In a 14 days' film the bacilli varied in length from ·75 to 2μ, the average being not over 1μ. They were uniformly stained. A few were curved.

On Broth.—Growth extremely poor. Minute flakes of growth appeared all over the surface in about a month, and in one flask, out of four inoculated, a small semi-translucent island had appeared at the end of two months. In a 56 days' film the bacilli varied in length from 1 to 3·5μ. A relatively small number were curved and beaded, the rest being straight and uniformly stained.

On Glycerin-Agar.—No more than a grey haze with a few minute grey points. In a 42 days' film the bacilli resembled those grown on serum.

On Potato.—A scanty, but uniformly distributed, white growth. In a 56 days' film the bacilli varied in length from 1 to 3·5μ. Most of them were straight; some were irregularly stained.

Summary.

Growth very poor.

(2).

(Human Cervical Gland.)

Strain.—Original Material.

Material Received.—Third generation, 13 days old, of culture isolated from human cervical gland.

On Bovine and Horse Serum.—Growth very poor at first, afterwards fairly satisfactory, but by no means good during the first five or six generations. In a 14 days' film, from bovine serum, the bacilli varied in length from ·75 to 1·5, occasionally 2μ, and were straight and uniformly stained.

On Broth.—With the exception of a few fine specks, no growth was observed during the first 6 weeks. (Four cultures tried.) Towards the end of the second month some thin, semi-opaque islands were formed. The largest of these was about 2 in. in diameter. In a 56 days' film the bacilli varied in length from 1 to 2·5 or 3μ. A few were curved and a few were beaded or irregularly stained.

On Glycerin-Agar.—No more than a fine grey haze was formed. In a 42 days' film the bacilli resembled those grown on serum.

On Potato.—A moderate number of small grey colonies were slowly formed. In a 56 days' film the bacilli varied in length from ·5 to $3{\cdot}5\mu$. Coccal forms were numerous. Most of the bacilli were uniformly stained. A few were curved.

Summary.

Growth very poor, like the strain isolated from the mesenteric gland but perhaps slightly better on broth.

(3).

(Human Lung.)

Strain.—Original Material.

Material Received.—Third generation, 13 days old, of culture isolated from the human lung.

On Bovine and Horse Serum.—In several generations the growth was poor; it then gradually improved. In a 14 days' film, on bovine serum, the bacilli varied in length from ·75 to 2μ. They were straight, but often showed globular swellings.

On Broth.—Growth extremely poor. At the end of a month a few minute islands were floating. Several more small, semi-translucent, fragmentary patches of growth appeared towards the end of the second month. In a 56 days' film the bacilli varied in length from 1 to $3{\cdot}5\mu$. The majority were straight. Many globular swellings were noted.

On Glycerin-Agar.—Growth very poor. At the end of two months there was a thin grey haze with a few denser points. In one culture a small, raised colony appeared at the margin between the culture medium and the glass. This colony, subcultured on to agar, produced an irregular, grey, somewhat granular growth which was much more abundant than the original culture. In a 39 days' film the bacilli resembled those grown on serum.

On Potato.—A few small white colonies were noted at the end of a month, and several more were observed at the end of the second month. In a 56 days' film the bacilli varied in length from 1 to 3μ. They were straight and uniformly stained.

Summary.

Growth very poor, like (2).

(4).

(Human Brain.)

Strain.—G.P. 1860 from Human Brain.

Material Received.—Second generation, 23 days old, of culture isolated from G.P. 1860, which had been inoculated with the human brain.

On Horse Serum.—Growth very poor at first; some improvement was noted after the first three or four subcultures, but the culture eventually died out. In a 14 days' film the bacilli varied in length from ·75 to $1{\cdot}5\mu$ (rarely 2μ), and were straight and uniformly stained.

On Broth.—Several flasks were inoculated but no growth was obtained. Allowance must be made for the fact that the serum cultures used for inoculation were not in an actively growing condition.

On Glycerin-Agar.—A slight grey haze only was formed. In a 42 days' film the bacilli resembled serum bacilli, with the exception of a few which were as long as 3μ and were regularly beaded.

On Potato.—No evidence of growth was obtained.

Summary.

Growth extremely poor.

VIRUS—H 61. E.C.

(1).

(Human Mesenteric Gland.)

Strain.—ORIGINAL MATERIAL.

Material Received.—Third generation, 13 days old, of culture isolated from the human mesenteric gland.

On Bovine and Horse Serum.—Growth good. In a 14 days' film, from bovine serum, the bacilli varied in length from ·75 to 1·5μ and averaged less than 1μ. They were straight and uniformly stained.

On Broth.—In a month the surface was covered with a grey layer, thick in many places but of unequal density. The pellicle showed no signs of becoming moist or sinking. In a 50 days' film the bacilli varied in length from 1 to 3·5μ. Curved and beaded forms were common.

On Glycerin-Agar.—The surface was readily covered with a uniform layer which gradually became dense, finely granular, and wrinkled. In a 39 days' film the bacilli varied in length from 1 to 3·5μ. They were mostly straight, or only slightly curved, and the staining was very uniform.

On Potato.—A cream-coloured, fairly abundant growth was obtained. In a 70 days' film the bacilli varied in length from 1 to 5μ. Curved and beaded forms predominated.

SUMMARY.

Fairly abundant growth.

(2).

(Human Brain.)

Strain.—G.P. 1837 from HUMAN BRAIN.

Material Received.—Second generation, 23 days old, of culture isolated from G P. 1837, which had been inoculated with the human brain.

On Horse Serum.—Growth good. In a 14 days' film the bacilli varied in length from ·75 to 1·5μ and were straight and uniformly stained.

On Broth.—Growth was slow, irregular, and of unequal density, but the pellicle did not become moist or show any tendency to sink. In a 28 days' film the bacilli varied in length from 1·5 to 3·5μ, and were uniformly stained. Curved forms were fairly numerous.

On Glycerin-Agar.—The surface was completely covered in a fortnight with a markedly wrinkled layer. The growth continued to increase in density and showed some small warty elevations. In a 42 days' film the bacilli varied in length from ·75 to 3 and occasionally 4μ, the average not being over 2μ. A few beaded forms were noted and some of the longer bacilli were curved, but otherwise the bacilli were straight and uniformly stained.

On Potato.—A brownish growth appeared at the end of the first week and afterwards became moderately dense. In a 56 days' film the bacilli varied in length from 1 to 3·5μ. They were uniformly stained, and about half were curved.

SUMMARY.

Growth good on glycerin-agar and potato, rather poor on broth.

VIRUS—H 62. W.M.

(Human Sputum.)

Strain.—G.P. 1786 from ORIGINAL MATERIAL.

Material Received.—Fourth generation, 11 days old of culture isolated from G.P. 1786.

On Bovine and Horse Serum.—Growth good. In a 12 days' film, from bovine serum, the bacilli varied in length from ·75 to 2μ and averaged not more than 1μ. They were straight and uniformly stained.

On Broth.—The surface was covered in about four weeks with a tough but unequally dense layer showing no tendency to sink. In a 22 days' film the bacilli resembled those grown on serum, but were slightly longer.

On Glycerin-Agar.—A wrinkled, granular layer was readily formed and ultimately became dense. In a 37 days' film the bacilli varied in length from ·75 to 3·5 or 4μ. The majority were uniformly stained, but some showed a tendency to beading. The longer forms were curved.

On Potato.—A fairly abundant, yellowish brown layer was formed. In a 56 days' film the bacilli varied in length from ·5 to 3·5μ, or occasionally 4 to 5μ. Coccal forms were frequent. The longer forms were curved. Staining was rather irregular.

SUMMARY.

Growth readily obtained and fairly abundant.

VIRUS—H 63. G.R.

(1).

(Human Mesenteric Gland.)

Strain.—Original Material.

Material Received.—Third generation, 17 days old, of culture isolated from the human mesenteric gland.

On Horse Serum.—Growth good. In a 13 days' film the bacilli varied in length from ·75 to 1·5μ (occasionally 2 to 2·5μ) and were straight and uniformly stained.

On Broth.—Growth was slow during the first three weeks, but the surface became completely covered in the sixth week. The pellicle was wrinkled, moderately dense, and showed no tendency to sink. In a 26 days' film the bacilli varied in length from 1 to 3·5μ. Most of them were straight; a small number were beaded.

On Glycerin-Agar.—The surface was rapidly covered with a grey layer which soon became wrinkled. At the end of six weeks the growth was dense and very prominently wrinkled. In a 52 days' film the bacilli varied in length from 1 to 5μ. The majority were curved. Beading and irregular staining were common.

On Potato.—A fairly abundant, pale yellow, crumb-like growth was formed. In a 56 days' film the bacilli varied in length from 1 to about 5μ. All except the shortest forms were curved and beaded.

Summary.

Growth abundant.

(2).

(Human Meninges.)

Strain.—G.P. 1877 from Human Meninges.

Material Received.—Second generation, 17 days old, of culture isolated from G.P. 1877, which had been inoculated with the human meninges.

On Horse Serum.—Growth good. In a 10 days' film the bacilli varied in length from ·5 to 1·5μ and were straight and uniformly stained.

On Broth.—Growth was slow, but led to the production of the thick, wrinkled type of pellicle, with no tendency to sink. In a 27 days' film the bacilli varied in length from ·75 to 3μ. They were uniformly stained and rarely curved.

On Glycerin-Agar.—The surface was covered with a grey layer which became very prominently wrinkled during the second and third weeks and afterwards grew denser. In a 52 days' film the bacilli varied in length from 1 to 6μ. Nearly all were curved. Regular beading was very common; irregular thickenings were also found, but were less common.

On Potato.—Growth was moderately abundant and was at first yellow but afterwards brown. In a 56 days' film the bacilli varied in length from 1 to 5 or 6μ and were nearly all curved and beaded.

Summary.

Growth similar to the strain from the mesenteric glands.

VIRUS—H 64. M.G.

(1).

(Human Mesenteric Gland.)

Strain.—G.P. 1910 from Human Mesenteric Gland.

Material Received.—Third generation, 9 days old, of culture isolated from G.P. 1910, which had been inoculated with the human mesenteric gland.

On Horse and Bovine Serum.—Growth poor for first three or four subcultures, afterwards satisfactory. In a 14 days' film from bovine serum the bacilli varied in length from ·75 to 2μ and were uniformly stained; a few were curved.

On Broth.—A thin but opaque pellicle covered about half the surface in three weeks. As the pellicle continued to increase, it became moist and sank. In a 30 days' film the majority of the bacilli resembled those grown on serum, but a few curved forms and a few with globular swellings occurred.

On Glycerin-Agar.—The surface became covered with a very scanty, greyish layer, which gradually exhibited some rather denser patches. In a 44 days' film the bacilli resembled those grown on serum.

On Potato.—A very scanty, white layer was obtained. In a 45 days' film the bacilli measured from ·75 to 3 μ. Most of them were uniformly stained; the longer forms were often curved.

Summary.

Growth poor on all media.

(2).

(Human Meninges.)

Strain.—G.P. 1881 from Human Meninges.

Material Received.—Third generation, 17 days old, of culture isolated from G.P. 1881, which had been inoculated with the human meninges.

On Horse Serum.—Growth poor. In an 18 days' film the bacilli varied in length from ·75 to 3, or occasionally 3·5μ. Numerous forms were noted which showed darkly-stained thickenings, the remainder of the bacilli being faintly stained. As these appearances are unusual for serum-grown bacilli a film from another culture was examined ; it was found to resemble the first.

On Broth.—Several flasks were inoculated, but no more than a few minute islands of growth were obtained. In a 56 days' film bacilli were scanty and did not occur in clumps. Some of them were fragmentary ; others were beaded or irregularly stained, often curved, and from 1 to 3·5μ in length.

On Glycerin-Agar.—A fine grey haze only was obtained. In a 48 days' film the bacilli resembled serum grown bacilli, but were rather longer. A few beaded forms occurred.

On Potato.—No definite growth obtained.

Summary.

Growth extremely poor ; poorer than the previous strain.

VIRUS-H 65. K.B.

(Human Mesenteric Gland.)

Strain.—G.P. 1835 from Human Mesenteric Gland.

Material Received.—Second generation, 17 days old, of culture isolated from G.P. 1835.

On Horse Serum.—Growth poor for first two or three generations, afterwards fairly good. In a 13 days' film the bacilli varied in length from ·75 to 2 and sometimes 2·5μ. Curved forms and beaded or irregularly stained forms were rather frequent.

On Broth.—The surface was almost covered in about a month with a delicate, partially transparent pellicle which afterwards became moist and sank. In a 27 days' film the bacilli varied in length from 1 to 3, occasionally 3·5μ. The majority were straight and irregularly stained.

On Glycerin-Agar.—In two months' time no more than a grey haze with a few small denser patches was formed. In a 52 days' film the bacilli varied in length from 1 to 3μ ; the great majority were straight and uniformly stained.

On Potato.—A few very scanty patches of white growth were found at the end of two months. In a 56 days' film the majority of the bacilli were short and straight (1 to 2·5μ) and somewhat irregularly stained. Here and there groups of longer bacilli were found (3 to 4μ) ; they were generally curved and uniformly stained.

Summary.

Growth very scanty.

THE BASIS OF CLASSIFICATION.

I now proceed to consider what basis for a classification of cultural characters is furnished by the preceding data.

The Influence of the Medium.

Serum.

Bovine Serum.—I have used for the cultures recorded above serum obtained from eighteen different bovines. These samples of serum have shown marked differences in their nutritive value. The samples of comparatively low nutritive value have all been obtained from calves; and, conversely, though different calf sera were not equal in nutritive value, in no instance was a calf serum of very high nutritive properties found. The samples of good serum were all obtained from adult animals; these, again, were not all equally good, but in no instance did an adult serum prove distinctly poor.

It is quite clear from my results that bovine serum is not a medium which differentiates those strains which I have grown upon it. They all grow on this medium, and it may be stated, subject to certain qualifications, that when a good sample of serum is used they all grow readily, when a poorer sample is used they all grow less readily. These qualifications are as follows:—First, with regard to the amount of growth. On several occasions the majority of my stock cultures have been subcultured, on the same day, on to a particular sample of bovine serum. On looking over the resultant growths I have found that those strains which grow well or fairly well on media other than serum, produce, on the whole, a denser growth on bovine serum than is formed by strains which grow poorly on media other than serum. Moreover, in dealing with bacilli recently isolated from the animal body, some strains take one or more generations longer than others before they have acquired the habit of growing well,, *i.e.* of covering the whole of the surface in about a fortnight with a moderately dense layer. The strains which take longer to acquire this habit are, generally, those which yield poor, or relatively poor, growths on media other than serum. Secondly, with regard to the character of growth. With the denser growths, colonies projecting above the general level are likely to be more frequent and larger, and pigmentation of the growth is much more common. From some samples of bovine serum many strains, particularly those which grow well on other media, are able to extract a bright yellow pigment.

Whilst these differences are noteworthy, it must be borne in mind that they are subject to many exceptions, that frequently they are not well marked, and that they are not always constant. It is, for example, frequently found that two strains which grow in every respect identically on bovine serum exhibit marked differences when tested on other media. Bacilli which grow well on other media will grow well on bovine serum; but the fact that bacilli grow well on bovine serum is no guarantee they will grow well on other media.

Microscopically, all the strains of bacilli are, when grown on bovine serum, practically alike.* They are, with slight exceptions and differences, short, straight, and uniformly stained. The most important factor, though not necessarily the only factor, on which the morphology of a tubercle bacillus depends is the nature of its nutritive material. The fact that bovine serum exerts practically the same influence on the morphology of all the strains of bacilli examined is, therefore, worth recording as a physiological feature which these strains possess in common.

Horse Serum.—I have used twenty-three samples of this medium. The nutritive value of these has been very nearly equal; it has been found to be below that of the best bovine serum, but well above that of the poorer bovine sera. With both bovine serum and horse serum obtained by allowing the blood to clot in the natural way a scum forms on the surface after the tubes have been coagulated. This scum, though partially removed when the tube is inoculated, is apt to re-form unless the organisms grow quickly. When the scum re-appears growth is delayed. This scum formation has given rather more trouble with the horse serum than with bovine serum. But with horse serum first obtained as plasma by means of potassium oxalate very little scum is formed.

On the whole, both the naked eye and the microscopic appearances of bacilli grown on horse serum are the same as those obtained with bovine serum.

Dog Serum.—The dog serum used for the cultures on which I have reported was a very favourable medium, perhaps slightly better than the best of my bovine sera. Practically all the strains inoculated grew well, the surface becoming covered with a thick layer and also exhibiting white, heaped-up colonies and patches. In the case of many of the strains, viz., those which grew with relative reluctance on media other than serum, this white, heaped-up appearance was often not observed in the first culture made on pure dog serum until the third week; but when, in these cases, the culture was passed through a second or third dog serum tube, this same appearance was observed earlier, generally at the end of the first week. The strains which grew well on media other than serum generally showed white, raised colonies at the end of the first week. As distinct from bovine serum, very little scum formation takes place on this medium. I do not find that dog serum is of any more use than bovine or horse serum as a differential medium. Morphologically, the bacilli resembled those grown on bovine and horse serum.

Pig Serum.—I have only used a small amount of this medium. The samples I used were in every respect equivalent to a fairly good bovine serum.

*Glycerinated Serum.**

I have always used bovine serum to which 5 per cent. of undiluted glycerin has been added. Compared with other glycerinated solid media such as glycerin-agar this medium has the advantage of producing growths more readily. But the characters of the growths obtanied are, with many viruses, less stable than is the case with glycerin-agar, and consequently they are less useful for purposes of differentiation. It often happened that a culture which had been making very little progress during the first week or the first fortnight would show a sudden and rather abundant increase subsequently; this increase often occurred during the third week. Again it was sometimes found that a culture which had been progressing rapidly during the first two or three weeks would fall off after about the third week, with the result that the ultimate yield proved to be much less than that observed on other cultures with which it had compared favourably during the earlier period of growth. The irregularity of the growths on this medium is also illustrated by comparison with control tubes containing serum which had been obtained from the same animal but to which no glycerin had been added. On referring to my notes on these comparisons it will be observed that the relative amounts of growth on the glycerinated tube and the pure serum tube varied very considerably from week to week. It often happened, for example, that the glycerinated culture was either inferior or not more than equal to the pure serum culture during the first fortnight, but surpassed the latter afterwards. An inspection of the tubes showed that this alteration in the relative amounts of growth was partly, at all events, accounted for by the fact that the glycerinated tubes retained a moister surface than the pure serum tubes. Owing to many irregularities, of which this is an example, I do not attach much importance to a comparison of glycerinated serum tubes with corresponding pure serum tubes. In the case of a great many strains it is quite impossible to say categorically that the strain grows either better or worse on glycerinated serum than on pure serum. I am therefore in complete disagreement with the opinion that comparisons such as these can be made the sole or the main criterion by which

* This subject is discussed more fully in the subsequent chapter on microscopic characters.

* The use of glycerinated serum has been recommended by Max Beck, who states (*Festschrift von Robert Koch*) G. Fischer: Jena, 1903) that the addition of glycerin favours the growth of "human" bacilli but retards the development of "bovine" bacilli. Kossel does not appear to attach much importance to this distinction, as he says, without further qualification, that bovine bacilli may be grown on artificial media, "especially on beef serum with or without addition of glycerine." (*British Medical Journal*, p. 1445. Dec. 2nd, 1905.)

the cultural characters of tubercle bacilli are to be differentiated.

On the whole, glycerinated serum, though a less satisfactory medium than glycerin-agar, confirms the results obtained by the latter. It is a medium which serves very well to illustrate the inaccuracy of the view that all viruses can be put into one of two classes, the class which grows well and the class which grows badly. Given an adequate number of viruses, it is possible to show that we can pass by a gradual and unbroken transition from those which grow very badly on glycerinated serum to those which grow extremely well. It is desirable to call attention to the fact that the cultural characters on glycerinated serum which I have recorded were obtained by inoculation on to this medium from strains which had not previously resided on any medium other than pure serum. I have found that by repeated subculture on glycerinated serum strains which at first grow poorly on this medium can be made to grow very much better.

Morphologically, the characters of bacilli from different strains vary widely and irregularly when grown on glycerinated serum, and do not provide any very reliable basis for a differentiation into types.

Broth.

5 per cent. glycerinated broth has always been used. My method of observing the readiness or reluctance of a culture to transfer itself from serum to broth has brought out marked differences between the different viruses. In some cases, after there was a good layer of growth on the serum, a delay of a week or two, or even longer, took place before there was any growth on the broth; and then the formation of a pellicle often began as small, isolated islands, which gradually increased in area and coalesced. In other cases, fair-sized patches of pellicle commenced to form as soon as the culture on the layer of serum touched the margin of the broth; and often a continuous layer of growth was seen streaming down the serum slope and spreading uninterruptedly across the surface of the broth. The character of the pellicle, its rate of increase, and its ultimate yield also varied with different viruses. Some grew slowly, with the formation of a very thin, semi-translucent pellicle which was sometimes homogeneous, sometimes speckled with a few small white patches, but never produced a copious or uniformly thick growth. In the case of a few of these reluctantly growing viruses the patch of pellicle first formed was uniformly thick, but, as it extended, it thinned out, and it never became uniformly dense. In the case of the more rapidly growing viruses the pellicle sometimes was at first thin, but afterwards became much denser; frequently a dense pellicle was formed from the commencement. Marked differences have also been noted in the floating capacities of the pellicles. With some strains the growth was rapid and moderately thick, but some became moist and then sank to the bottom. This tendency to sink readily was not exhibited by the dense, rough, and wrinkled types of pellicle.

The morphological characters of the bacilli varied with different strains. It has not been possible to make out any correspondence between the macroscopic and the microscopic features of broth cultures which is sufficiently constant to provide a reliable basis for differentiation.

Glycerin-Agar.

Very marked cultural differences have been brought out by the use of this medium. The following is a brief outline of the different types of growth which have been observed. (1) During the first five or six weeks the growth consists of no more than a fine grey haze on the surface; towards the end of the second month a few large, isolated colonies may slowly form, originating, apparently, from the relatively larger particles of material originally inoculated. (2) An opaque, scanty, often moist, grey layer is formed at the end of three weeks, but fails to make much progress subsequently, the growth always remaining thin and generally patchy. (3) The surface is completely covered with a somewhat dense, grey layer during the first fortnight; growth then continues, somewhat slowly, until about the sixth week; the total yield is moderately dense, sometimes moist, and sometimes with a dry, granular appearance on the surface. (4) Growth is rapid and spreads as a uniform, thick layer all over the surface. After about a fortnight the layer of growth becomes wrinkled and the wrinkles increase in prominence as the growth continues. (5) Growth still more abundant; the layer is both wrinkled and thrown into warty elevations.

Miscroscopically the characters of the bacilli vary very considerably with different strains, and cannot be relied upon as a stable basis for differentiation.

Potato.

Marked differences are brought out which correspond in a general way to the differences demonstrable on glycerin-agar. The types of growth may be, roughly, grouped into three: (1) At the end of about five weeks there is so little to be seen that it is doubtful if there has been any increase of the material inoculated. (2) At the end of the same period there is a clearly recognisable, though scanty, increase, without any pigment formation. (3) In from one to two weeks' time yellow colonies appear; copious increase then takes place; and in five weeks the cultures have already, sometimes, assumed a typical brownish-yellow, pulled bread appearance. As on glycerin-agar, the microscopic appearances of the bacilli vary considerably and exhibit no invariable relationship to the nature or amount of growth.

Media of Differential Value.

From the above considerations it is clear that the three media which are of marked differential value are broth, glycerin-agar, and potato. Of these, I regard glycerin-agar as the most important, and potato as the least; and I think that the three taken together help to control each other, and therefore give more reliable information than any one taken alone.

As a basis for classification, therefore, I take the combined characters which my cultures exhibit on broth, glycerin-agar, and potato.

According to this principle, I have arranged my strains in order, according to their capacity for growth on these media, and commencing the series with those strains which grow least favourably.

I attach high importance to the fact that, when arranged in this way, my strains present one continuous and absolutely unbroken series, passing by gradual and only just perceptible stages of transition from the top of the series, which contains the strains which grow with greatest difficulty, down to the bottom of the series where are found the strains which grow with greatest luxuriance.

This series I have drawn up in tabular form (pp. 228-232).

THE CHARACTERISTICS OF INDIVIDUAL VIRUSES.

B I.

Strains : (1) *G.P.* 14 ; (2) *Cow* 44 ; (3) *Calf* 122.—These three strains are typical of Grade I. Cow 44 grows rather better than the other two. My investigations commenced with the fourteenth generation of G.P. 14, the third generation of Cow 44, and the third generation of Calf 122. It is noteworthy that prolonged residence on serum has not increased the growing capacity of G.P. 14.

Strains : (4) *Baboon* 8 ; (5) *Baboon* 64 ; (6) *Baboon* 66. —The culture from Baboon 8 shows a decided increase in luxuriance, being classed in Grade III. This modification appears, however, to have not been of a permanent nature, since Baboon 64 had been fed with ·1 mg. of culture of Baboon 8 and the culture obtained from Baboon 64 is classed in Grade 1. The culture from Baboon 66, derived from Cow 44 through Monkey 76, is also classed in Grade I.

Strains : (7) *Monkey* 80 ; (8) *Monkey* 82.—These animals were fed with minute doses of culture from Cow 44. The strains resemble on the whole Cow 44 but grow slightly better.

Strains : (9) *Pig* 8 ; (10) *Pig* 96 ; (11) *Pig* 98.—Pig 8 was fed with the infected milk of Cow 40 ; the culture is classed amongst the better growing organisms at the bottom of Grade I. From Pig 8 the virus was passed through Pig 12 and thence through Pig 16. Pigs 96 and 98 were both inoculated with a culture from Pig 16. The culture from Pig 96 grows less readily than the culture from Pig 8 being no better than the culture from G.P. 14. On the other hand the culture from Pig 98 grows slightly better than the culture from Pig 8, being classed in the upper part of Grade II.

Conclusions.—Of the eleven strains examined, eight are placed in Grade I., two in Grade II., and one in Grade III. The virus as a whole exhibits some variability in its cultural characters ; but these variations are too inconstant to be regarded as evidence of permanent modification due to the specific action of the tissues of the animal in which the bacilli have resided. There is no indication that either the pigs or the monkeys have exercised a specific influence on the cultural characters of the bacillus, and the case of Baboon 8 is the only instance where the bacilli have shown a large increase of luxuriance after residence in the tissues of baboons.

B II.

Strains : (1) *Heifer* 100 ; (2) *Calf* 134 ; (3) *G.P.* 1735.— There three strains are very much alike and are all classed in Grade I. The strain through G.P. 1735 had resided in guinea-pigs for a period of 40 months, without any change in cultural characters being produced.

B III.

Strains : (1) *Original Material ;* (2) *Mouse* 10 ; (3) *Monkey* 74.—All three strains are placed in Grade I. The bacilli show no change after residence in the mouse. Monkey 74 grows slightly better than the other two strains.

B IV.

Strains : (1) *Original Material ;* (7) *Cow* 172.—These two strains are very much alike, and are both placed in Grade 1.

Strains : (2) *Rat* 42 ; (3) *Cat* 12.—Both these strains are placed in Grade 1. Neither the rat nor the cat appears to have produced any important modification in the characters of the bacilli.

Strains : (4) *Dog* 18 ; (5) *Dog* 92 ; (6) *Dog* 116.—Dog 18 is classed in Grade IV ; Dog 92 in Grade III. ; and Dog 116 in Grade II. There is therefore a very decided increase of luxuriance after residence in the dog, though I have not found this increase so well marked with Dog 116 as with the other animals. All three dogs were used for feeding experiments. Dog 18 was fed with bacilli from Cow 164 ; Dog 92 was fed with culture recovered from a collodion capsule which had been inserted in Dog 54 ; and Dog 116 was fed with culture from Dog 74 ; Dog 74 had been inoculated subcutaneously with a culture from Dog 56 (a collodion capsule experiment).

Strains : (8) *Baboon* 22 ; (9) *Baboon* 62.—Both these strains are placed in Grade I and show no important difference from the original material.

Strains : (10) *Chimpanzee* 4 ; (11) *Chimpanzee* 6 ; (12) *Chimpanzee* 8 ; (13) *Chimpanzee* 10 ; (14) *Chimpanzee* 16. —All these strains are classed in Grade I. With some of them the growth is rather poorer than with the original material.

Conclusion : Marked increase in luxuriance has been observed after residence in the dog ; but no change in this direction has been produced by the rat, cat, baboon, or chimpanzee.

B V.

Strains : (1) *Rabbit* 11 ; (2) *Goat* 2 ; (3) *Heifer* 80 ; (4) *Dog* 80. All these strains are classed in Grade II and exhibit only slight differences from one another. Neither the rabbit, goat, nor dog appear to have exercised any modifying influence.

B VI.

Strains : (1) *Original Material ;* (2) *Calf* 240.—Both strains are placed in Grade I ; the growth of Calf 240 is the better of the two.

B VII and B VIII.

Both these viruses are classed in Grade II.

B IX.

Strain : (1) *Original Material.*—This strain grows with a considerable degree of luxuriance, being one of the better growing viruses classed in Grade III.

Strains : (3) *Cat* 52 ; (8) *Goat* 32 ; (9) *Hedgehog* 22 ; (11) *Mongoose* 14 ; (13) *Pig* 108 ; (14) *Rabbit* 347.—All these strains are classed in Grade III. ; they do not exhibit more than slight differences from one another or from the original material.

Strains : (2) *Baboon* 68 ; (4) *Chimpanzee* 14 ; (10) *Lemur* 10 ; (12) *Monkey* 116.—These strains, again, are classed in Grade III. and resemble the above mentioned strains.

Strains : (5) *Dog* 50 ; (6) *Dog* 60 ; (7) *Dog* 84.—Dogs 60 and 84 are both classed in Grade III. but Dog 60 grows slightly better than any of the above mentioned strains. Dog 50 is classed amongst the most luxuriant strains placed in Grade IV. ; the increase in luxuriance is therefore very marked. There was no special reason to suspect contamination by spontaneous infection in this case, but as I have not, hitherto, met with any other bovine strain which grows so luxuriantly as this, I am not prepared to lay much emphasis on it as an instance of modification by the dog. It is interesting to note, however, that Dog 18 (B IV.), though not quite so luxuriant, is also classed in Grade IV.

Conclusions.—With the exception of Dog 50, which requires corroboration, all the strains of B IX. retain their original characteristics with remarkable tenacity.

B X. and B XI.

Both these viruses are classed in Grade III.

B XII.

Strains : (1) *G.P.* 1159 ; (2) *Rabbit* 98.—The former strain grows very poorly, being one of the least readily growing organisms classed in Grade I. The latter strain grows appreciably better, being classed in Grade II. This difference may perhaps be attributed to the influence of the tissues of the rabbit.

B XIII.

Strain : Rabbit 116.—This strain is classed amongst the least readily growing organisms placed in Grade I. The rabbit has not, therefore, favoured luxuriance of growth in this case.

B XIV. to B. XXI.

These viruses exhibit no features of special interest. Three of them, *viz.*, B XIV., B XVI., and B XX. are classed in Grade II. ; the rest in Grade I.

B XXII.

Strains : (1) *Original Material* ; (2) *Dog* 72.—The former strain is placed in Grade I. ; the latter in Grade III. This is a well marked instance of increased luxuriance after residence in the dog.

B XXIII. to B XXVII.

These viruses present no features of special interest. B XXV. and B XXVI. are classed in Grade II., the others in Grade I.

B XXVIII.

The only special feature about this virus is that it was obtained from butcher's meat. It is classed in Grade II.

B XXIX. and B XXX.

B XXIX. is classed in Grade III. and B XXX. in Grade I.

H 2. Sp. A.

Strains : (1) *Calf* 93 ; (2) *Calf* 111.—Both these strains are classed amongst the better growing cultures in Grade III. and approximate closely to Grade IV. Of the two Calf 111 grows rather better. This virus is variable in its mode of growth. It has often been observed with cultures inoculated from the same material that some grow much better than others. Moreover, cultures of Calf 93, when inoculated into calves, have proved of much higher virulence than cultures of Calf 111. These apparent discrepancies are no doubt explained by the fact that the original material was a mixture, consisting of sputum from various cases of human phthisis, and therefore probably containing bacilli of different degrees of virulence.

H 7. C.M.

Strains : (1) *Cow* 73 ; (2) *Calf* 103.—These two strains grow alike and are typical of Grade III. The virus, which was obtained from human mesenteric glands, is highly virulent for bovines.

H 8. S.C.

Strains : (1) *Original Material ;* (2) *Calf* 361.—Both these strains are classed in Grade IV., but Calf 361 does not grow quite so well as the other strain. In Calf 361 disseminated tuberculosis, with the formation of typical retrogressive tubercles, had been produced after intravenous inoculation.

H 9. C.T.

The growth of this virus is typical of Grade IV.

H 10. B.S.

Strains : (1) *Original Material ;* (2) *Heifer* 231 ; (3) *Goat* 3.—These three strains closely resemble each other and are classed in Grade I. There is no evidence of modification either by the bovine or the goat.

H 11 E.D.

This virus grows abundantly and is classed in Grade V.

H 12. H.N.

Strains : (1) *Original Material ;* (2) *Rabbit* 640.—Both these strains are classed in Grade V. Rabbit 640 is rather the better grower of the two. This strain had resided within the tissues of rabbits for 19½ months. The rabbit, therefore, has been unable to produce any important modification.

H 13. A.D.

Strain : (1) *Calf* 301.—The bacilli grow well, being classed in Grade IV. They had produced very severe disease in the animal from which they were obtained. But cultures of this strain, inoculated in 50 mg. doses, failed to produce fatal infection in calves in 90 days.

Strains : (2) *Rat* 15 ; (3) *Calf* 321.—Both these strains grow very poorly and are classed in Grade I. Both Rat 15 and Calf 321 had been inoculated from Calf 301. There has therefore been a very marked modification in cultural characters.

H 14. F.S.

Strains : (1) *Original Material ;* (2) *Calf* 125 ; (3) *Calf* 327 ; (4) *Calf* 895.—These strains grow very much alike. They are classed amongst the better growing organisms in Grade I., with the exception of Calf 895 which is slightly better and is placed in Grade II.

H 15. I.W.

This virus grows abundantly and is classed in Grade V.

H 16. J.H.

Strains : (1) *Calf* 157 ; (2) *Calf* 273.—Both these strains are classed in Grade V.

Strains : (3) *Calf* 337 ; (4) *Calf* 355.—Calf 337 is classed in Grade III. and therefore shows a marked diminution in luxuriance compared with the two previous strains. Calf 337 was inoculated from Calf 273. The culture from Calf 355, another animal inoculated from Calf 273, is also classed in Grade III.

Strains : (5) *Calf* 423A ; (6) *Calf* 559.—Calf 423A is classed in Grade I. ; there is therefore again a further and very marked diminution in luxuriance. Calf 423A was inoculated from Rabbit 66, which had been inoculated from Calf 355. Calf 559 is classed in Grade III. This animal was inoculated with a culture of Calf 423A. The bacilli have therefore increased in cultural luxuriance after residence in Calf 423A.

Conclusions.—The cultural characters of this virus were originally those of Grade V., but after repeated passage in the bovine they have been modified to those of Grade III. and Grade I. With this modification may be compared the change in virulence. The cultures of Calf 273 proved of low virulence to the bovine when inoculated in 50 mg. doses, whilst the cultures of Calves 337 and 423A when tested similarly, proved to be of high virulence. As illustrating the instability or capacity for modification of the bacilli, it is interesting to note that in Calf 559 they again reverted from the Grade I. to the Grade III. type.

H 17. Sp. B.

Strain : (1) *Calf* 265.—This culture is classed in Grade V. It is of low bovine virulence, but of no special interest, as experiments were not continued with it.

Strain : (2) *Calf* 339.—This culture is classed amongst the better-growing organisms in Grade IV. and is important since *Calf* 339 formed the commencement of a modification experiment.

Strain : (3) *Rabbit* 181. This culture is also classed in Grade IV. and resembles Calf 339. The intermediate passages between Calf 339 and Rabbit 181 are as follows :—(1) Guinea-pigs ; (2) Calf 475 ; (3) Calf 539.

Strain : (4) *Calf* 553.—Calf 553 is classed in Grade I., and therefore shows a very marked diminution in cultural luxuriance compared with the preceding strains. Calf 553 was inoculated from Calf 529, and Calf 529 had been inoculated from Calf 539.

Strain : (5) *Calf* 555.—This is also classed in Grade I., and grows even more poorly than Calf 553. Calf 555 was inoculated from Calf 553.

Strain : (6) *Calf* 571.—This strain is identical with Calf 555. Calf 571 was inoculated from Calf 557, and Calf 557 had been inoculated from Calf 553.

Conclusions.—This series therefore exhibits a modification of cultural characters from Grade IV. to Grade I. The steps in the modification of virulence for bovines, tested in 50 mg. doses of culture, were as follows. The Calf 339 cultures were found to be of low virulence, but the cultures from Rabbit 181, Calf 553, and Calf 571 all proved to be of high virulence. It therefore appears, from Rabbit 181, that the increase in virulence took place before the modification in cultured characters.

H 18. T.T.

This virus is typical of Grade IV.

H 19. S.W.

Strains: (1) *Heifer* 239; (2) *Goat II.*—These two strains resemble each other and are placed in Grade III.

H 20. F.L.

This culture is classed in Grade II.

H 22. F.W. and H 23 J.P.

These viruses are typical of Grade V.

H 25. A.T.

Strains: (1) *Original Material;* (2) *Chimpanzee* 1; (3) *Chimpanzee* 3; (4) *Dog* 1.—All these strains grow well, but not quite equally well. (1) and (3) are classed in Grade V., (3) being the better of the two; (2) and (4) are placed in Grade IV.

H 26. K.M. and H 27. B.D.

These viruses belong to Grade V.

H 28. C.L.

Strains: (1) *Original Material;* (2) *Monkey* 63.—These strains resemble one another and are placed in Grade I. There is no sign of modification by the monkey.

H 29. M.F.

This virus belongs to Grade II.

H 30. E.M.

This virus belongs to Grade IV.

H 31. L.F.

This virus belongs to Grade II.

H 32. Y.W.

Strains: (1) *from Human Bronchial Gland;* (2) *Human Mesenteric Gland.* These strains closely resemble each other. They both are placed in Grade III.

H 33. R.T.
H 34. C.U.
H 35. C.B.
H 36. M.D.
H 37. O.J.

These viruses present no features of special interest. 33 and 37 are placed in Grade V., the others in Grade IV.

H 38. J.M.

This virus is placed in Grade III.

H 39. M.B.
H 40. J.G.
H 41. A.S.
H 42. M.R.
H 43. F.F.
H 44. D.C.
H 45. F.M.
H 46. H.W.
H 47. S.B.
H 48. W.P.

These viruses are not of special interest, 39 and 42 are placed in Grade IV., the rest in Grade V.

H 49. T.C.

Strains : (1) *Original Material :* (2) *Calf* 787 : (3) *Calf* 797.—(1) and (3) are classed in Grade II., (2) grows somewhat better and is placed in Grade III.

I find no difference between the cultural characters of Calf 797 and strain (1). Calf 787, however, which grows slightly better, was the companion animal to 797.

This virus was obtained from the mesenteric glands of a young adult. The virulence of strain (1) was at first high for rabbits, but afterwards became very much diminished.

The virulence of this strain for bovines was tested upon five calves in doses of 50 mg. After two months' cultivation, Calves 787 and 797 were inoculated; the former was fatally infected in 81 days, the latter was killed, with only a slight amount of disease, in 83 days. After seven months' cultivation, strain (1) was inoculated into Calves 957 and 959 ; both were fatally infected, the former in 61 and the latter in 53 days. The strain, therefore, proved more virulent after continued residence on culture. A possible explanation of this paradoxical result is that in the earlier inoculations the bacilli happened to be in a not very vigorous condition. After fourteen months cultivation, the same strain was again inoculated but into one calf only (1097). This animal was killed, with only a slight amount of disease, in 89 days, and therefore repeated the result obtained with Calf 797 (inoculated with the two months' culture). Taken alone, the one experiment with the 14 months' culture is obviously inconclusive ; but, taken in conjunction with the fact that the virulence of the strain had become diminished for rabbits, it appears to indicate that other calves, had they been inoculated, would not have been fatally infected.

The virulence of strain (3), (Calf 797), was also tested upon bovines ; it proved highly virulent in doses of both 50 mg. and 10 mg.

Taken as a whole, therefore, these results indicate that this virus is of a somewhat unstable character. But beyond this, they do not carry us very far. They are too irregular, and not sufficiently weighty, to justify any important inference.

H 50. P.H.
H 51. H.M.
H 52. T.F.

H 51 is classed in Grade V ; the other two viruses in Grade IV.

H 53. D.H.

Strains : (1) *Original Material ;* (2) *Calf* 905.—Both these strains are classed in Grade III., but (2) grows better than (1). Compared with other viruses classed in Grade III., the virulence of this virus for bovines is unusually low ; the virulence for rabbits, however, appears to be high. The virus was derived from a case of lupus.

H 54. C.W.

This virus is classed in Grade V.

H 55. R.D.

Strains : (1) *Human Bronchial Gland ;* (2) *Human Mesenteric Gland.*—Both grow readily but (1) is the better of the two, being classed in Grade V., whilst (2) is classed in Grade IV.

H 56. F.T.

This virus is classed in Grade V.

H 57. B.J.

Strains : (1) *Human Lung ;* (2) *Human Mesenteric Gland ;* (3) *Human Liver.*—These three strains are all classed in Grade V. ; (1) grows the best of the three.

H 58. F.G.

Strains.—(1) *Human Cervical Gland* ; (2) *Human Lung.*—The former strain is classed in the bottom of Grade III., the latter in the upper part of Grade IV. Neither strain has proved of high virulence for bovines.

H 59. L.B.

Strains.—(1) *Human Mesenteric Gland* ; (2) *Human Cervical Gland* ; (3) *Human Lung* ; (4) *Human Brain.*—All these strains grow very poorly and are classed in Grade I. ; strain (4) is the poorest of the four. All the strains have proved highly virulent for bovines.

H 61. E.C.

Strains.—(1) *Human Mesenteric Gland* ; (2) *Human Brain.*—Both these strains grow well and are classed in Grade IV. They have been found of low virulence for bovines.

H 62. W.M.

This virus is classed in Grade IV.

H 63. G.R.

Strains.—(1) *Human Mesenteric Gland* ; (2) *Human Meninges.*—Both these strains are placed in Grade IV. They have both been found of low virulence for bovines.

H 64. M.G.

Strains.—(1) *Human Mesenteric Gland* ; (2) *Human Meninges.*—Both these strains are classed in Grade I. ; (1) grows rather better of the two. Both are highly virulent for bovines.

H 65. K.B.

This virus (mesenteric gland) is classed in Grade I. It has been found of high virulence for bovines.

CLASSIFICATION AND COMPARISON OF BOVINE AND HUMAN CULTURES.

The following classification is based on the combined characters which my cultures exhibit on broth, glycerin-agar, and potato.

As my series makes a very lengthy list, I have divided. it up, for the purpose of convenience, into five grades This subdivision makes it easy to indicate approximately the position in my series of any particular strain, by referring to it as belonging to a certain grade. Within each grade, again, there are differences, the strains at the top growing less well than those at the bottom. My strains have become so numerous that I often find several about which it is impossible to say that one grows better or worse than another. Such strains I group together, arranging them according to the numerical order of the viruses to which they belong. Each group is marked off from the following slightly better growing group by a line drawn across the page.

The bovine and the human viruses are placed in parallel columns, strains with identical cultural characters being opposite each other.

GRADE I.

General Characters.—Growth poor on all three media. On broth the pellicle is often slow in making its appearance ; it is generally very delicate, semi-translucent and speckled with a variable number of white spots ; occasionally it is opaque ; it is very thin on the whole, and, with the exception of a few irregularly thickened areas, uniform. On glycerin-agar, a thin grey haze on the surface is generally all that is to be seen at the end of five or six weeks. On potato, at the end of the same period, the growth does not consist of more than a few minute grey colonies or streaks.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original human material.
I.	G.P. 14 from original material	Calf 321	13 A.D.	Bronchial glands and spleen
I.	Calf 122	Rat 15 from Calf 301	13 A.D.	Bronchial glands and spleen
I.	Pig 96			
III.	Original material	Calf 423A	16 J.H.	Knee joint
III.	Mouse 10 from original material	Calf 555	17 Sp. B	Mixed sputum
IV.	Chimpanzee 8	Calf 571	17 Sp. B	Mixed sputum
IV.	Chimpanzee 10			
IV.	Chimpanzee 16			
VI.	Original material	Original material	28 C.L.	Cervical glands
XII.	G.P. 1159 from original material	Monkey 63	28 C.L.	Cervical glands
XIII.	Rabbit 116 from original material	G.P. 1860 from original material	59 L.B.	Brain
XIX.	Original material	G.P. 1881 from original material	64 M.G.	Meninges
XXI.	Original material			
XXIII.	Original material			
XXIV.	Original material			
II.	Heifer 100			
IV.	Cat 12 from Cow 164			
IV.	Baboon 22			
IV.	Chimpanzee 4			
IV.	Chimpanzee 6			
XV.	G.P. 1348 from original material			
XXII.	G.P. 1509 from original material	G.P. 771 from original material	10 B.S.	Mesenteric glands
XXIV.	G.P. 1548 from original material	G.P. 1850 from original material	59 L.B.	Mesenteric gland
		Original material	59 L.B.	Cervical gland
		Original material	59 L.B.	Lung
XXX.	Original material			
I.	Cow 44			
I.	Baboon 64			
I.	Baboon 66			
III.	Monkey 74.	Heifer 231	10 B.S.	Mesenteric glands*
IV.	Original material	G.P. 798 from Goat 3	10 B.S.	Mesenteric glands
IV.	Baboon 62			
XVII.	Original material	G.P. 1542 from Calf 553	17 Sp. B.	Mixed sputum
XVIII.	Original material	G.P. 1835 from original material	65 K.B.	Mesenteric gland
I.	Monkey 82	G.P. 587 from original material	14 F.S.	Mesenteric glands
I.	Pig 8	Calf 125	14 F.S.	Mesenteric glands
II.	Calf 134	G.P. 1108 from Calf 327	14 F.S.	Mesenteric glands
II.	G.P. 1735 from series of G.P.'s.			
IV.	Rat 42 from original material	G.P. 1910 from original material.	64 M.G.	Mesenteric glands
IV.	Cow 172			
VI.	Calf 240			
XXVII.	Original material			

GRADE II.

General Characters.—Growth better than in Grade I., but not copious on any of the three media. On broth the pellicle often commences to form rather early and grows fairly quickly during the first fortnight after its appearance ; it often exhibits dense patches, but is never uniformly dense ; it very readily becomes moist and falls to the bottom. On glycerin-agar, at the end of three weeks, there is a greyish, moist, scanty, slightly raised growth which does not make much progress subsequently. On potato the growth is poor, non-pigmented and not at all abundant, but rather better than in Grade I. ; the first signs of growth generally appear earlier.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original human material.
I.	Monkey 80	Calf 895	14 F.S.	Mesenteric glands
		G.P. 1329 from original material	29 M.F.	Cervical glands
I.	Pig 98			
IV.	Dog 116			
VII.	G.P. 1017 from original material			
VIII.	Original material			
XII.	Rabbit 98 from original material			
XIV.	Original material			
XVI.	Original material			
XX.	G.P. 1471 from original material			
XXVI.	Original material			
XXVIII.	Original material			
V.	Heifer 80			
XXV.	Original material	Original material	49 T.C.	Mesenteric glands
		Calf 797	49 T.C.	Mesenteric glands
V.	Rabbit 11 from original material	Calf 213	20 F.L.	Mesenteric glands
V.	Dog 80 from original material			
V.	Goat 2	Original material	31 L.F.	Cervical glands

GRADE III.

General Characters.—On broth the surface is generally covered more quickly than in Grade II. and the pellicle formed is sometimes, but not invariably, thicker ; it very often exhibits a tendency to break up into islands which readily become moist at their margins, and then soon sink to the bottom. On glycerin-agar the growth is sometimes in raised, moist patches and sometimes dry and of the frosted glass type ; as we descend towards the bottom of the grade, it becomes fairly dense but not wrinkled. On potato a moderate, but not a good, yield is attained ; it is occasionally slightly pigmented.

Bovine Viruses.		Human Viruses.		
Number of Viruses.	Strain.	Strain.	Designation of Virus.	Original human material.
I.	Baboon 8	Calf 787	49 T.C.	Mesenteric gland
IX.	Cat 52			
IX.	Chimpanzee 14			
IX.	Goat 32			
IX.	Lemur 10			
IX.	Mongoose 14			
IX.	Monkey 116			
IX.	Pig 108			
IX.	Rabbit 347			
IX.	Original material	Calf 337	16 J.H.	Knee joint
		Calf 355	16 J.H.	Knee joint
IX.	Baboon 68	Heifer 239	19 S.W.	Mesenteric glands
IX.	Dog 84 from Dog 60	Goat 11	19 S.W.	Mesenteric glands
		Original material	32 Y.W.	Mesenteric glands
IX.	Hedgehog 22	Rabbit 166 from original material	32 Y.W.	Bronchial gland
X.	G.P. 1070 from original material	G.P. 793 from original material	38 J.M.	Cervical gland
XI.	Rabbit 66 from original material	G.P. 1482 from original material	53 D.H.	Lupus
XXIX.	Original material			
IX.	Dog 60	Calf 93	2 Sp. A.	Mixed Sputum
XXII.	Dog 72	G.P. 552 from Cow 73	7 C.M.	Mesenteric glands
		G.P. 640 from Calf 103	7 C.M.	Mesenteric glands
		Calf 559	16 J.H.	Knee joint
IV.	Dog 92 from Dog 54	G.P. 690 from Calf 111.	2 Sp. A.	Mixed Sputum
		Calf 905	53 D.H.	Lupus
		G.P. 1816 from original material	58 F.G.	Cervical gland

GRADE IV.

General Characters.—On broth the growth is, on the whole, more vigorous than in Grade III. and, ultimately denser ; it does not exhibit any tendency to become moist and sink. On glycerin-agar the surface is readily covered with a uniform layer, which afterwards becomes wrinkled ; but though the wrinkled appearance often becomes very accentuated the growth does not usually become very dense, heaped up, or warty. On potato the growth is moderately good ; somewhat heaped up, and slightly pigmented as a rule.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original human material.
IV.	Dog 18 from Cow 164	Calf 361	8 S.C.	Mesenteric glands
		Calf 301	13 A.D.	Bronchial gland and spleen
		G.P. 999 from Calf 131	18 T.T.	Mesenteric glands
		Calf 405	18 T.T.	Mesenteric glands
		G.P. 236 from original material	34 C.U.	Cervical glands
		G.P. 1571 from original material	36 M.D.	Lung and mesenteric gland
		G.P. 1567 from original material	52 T.F.	Lung
		Original material	55 R.D.	Mesenteric gland
		G.P. 1812 from original material	58 F.G.	Lung
IX.	G.P. 1708 from Dog 50	Original material	8 S.C.	Mesenteric glands
		G.P. 996 from Calf 183.	9 C.T.	Wrist
		Calf 339	17 Sp. B.	Mixed sputum
		Rabbit 181 from Calf 539	17 Sp. B.	Mixed sputum
		Chimpanzee 1	25 A.T.	Lung
		G.P. 1833 from Dog 1	25 A.T.	Lung
		G.P. 1338 from original material	30 E.M.	Mesenteric and bronchial glands
		G.P. 571 from original material	35 C.B.	Bronchial glands
		Original material	39 M.B.	Cervical glands
		G.P. 1447 from original material	42 M.R.	Ankle joint
		G.P. 1490 from original material	50. H.P.	Lung
		Original material	61 E.C.	Mesenteric gland
		G.P. 1837 from original material	61 E.C.	Brain
		G.P. 1786 from original material	62 W.M.	Sputum
		Original material	63 G.R.	Mesenteric gland
		G.P. 1877 from original material	63 G.R.	Meninges

GRADE V.

General Characters.—Growth luxuriant on all three media. On broth, rapid formation of thick, tough, wrinkled pellicle, with no tendency to sink. On glycerin-agar, a layer which is usually both warty and wrinkled and denser than in Grade IV. On potato, rapid formation of a heaped-up, richly pigmented growth.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original human material.
		G.P. 918 from Calf 221	11 E.D.	Elbow joint
		G.P. 577 from original material	12 H.N.	Mesenteric gland
		Calf 311	15 I.W.	Ankle joint
		Calf 157	16 J.H.	Knee joint
		Calf 273	16 J.H.	Knee joint
		Calf 293	22 F.W.	Lung and bronchial gland
		Calf 365	23 J.P.	Lung
		G.P. 1282 from original material	25 A.T.	Lung
		G.P. 1439 from original material	40 J.G.	Testis
		Original material	44 D.C.	Cervical gland
		Original material	51 H.M.	Lung
		G.P. 1609 from original material	54 C.W.	Bronchial gland
		Original material	55 R.D.	Bronchial gland
		G.P. 1682 from original material	56 F.T.	Lung
		G.P. 1802 from original material	57 B.J.	Mesenteric gland
		G.P. 1807 from original material	57 B.J.	Liver
		Rabbit 640	12 H.N.	Mesenteric gland
		Calf 265	17 Sp.B.	Mixed sputum
		Chimpanzee 3	25 A.T.	Lung
		G.P. 1326 from original material	26 K.M.	Kidney
		G.P. 1216 from original material	27 B.D.	Cervical gland
		Original material	33 R.T.	Axillary gland
		G.P. 1408 from original material	37 O.J.	Cervical gland
		G.P. 1437 from original material	41 A.S.	Knee joint
		G.P. 1425 from original material	43 F.F.	Knee joint
		G.P. 1137 from original material	45 F.M.	Bronchial gland
		G.P. 1444 from original material	46 H.W.	Knee joint
		G.P. 1430 from original material	47 S.B.	Hip joint
		G.P. 1494 from original material	48 W.P.	Lung
		G.P. 1800 from original material	57 B.J.	Lung

ANALYSIS OF RESULTS.

I call attention to the following features of importance which are exhibited in my classification of the cultural characters of bovine and human viruses.

Strains not modified by Animal Passage.

1. Thirty different bovine viruses have been investigated. In the case of several of these, more than one strain or sample has been examined. The strains have been derived sometimes directly from the original material of the naturally infected bovine which formed the starting-point of the virus, sometimes from experimental animals inoculated with this original material, or with cultures of it, and sometimes from experimental animals inoculated with bacilli which had previously resided in other experimental animals. The total number of strains examined is seventy-seven. Of these strains five showed definite evidence of modification as the result of experimental animal passage, *viz.*, Baboon 8 (B I), Dog 72 (B XXII), Dog 92 (B IV), Dog 18 (B IV), and Dog 50 (B IX). Of the remaining seventy-two none showed marked evidence of experimental modification*. These seventy-two strains, therefore, may be taken as representative of the natural, unmodified cultural characters of the thirty bovine viruses.

2. Fifty-seven human viruses have been investigated, and in several instances more than one strain of the same virus has been dealt with. The strains have been derived sometimes directly from the human material, and sometimes, as with the bovine strains, from experimental animals. The total number of strains examined is ninety-eight. Of these strains, eleven, belonging to the viruses H 13, A.D., H 16, J.H., and H 17, Sp. B., showed definite evidence of modification as the result of animal passage. The remaining eighty-seven are representative of the natural cultural characters of the bacilli originally resident in the human tissues.

3. There are, therefore, available, for the purpose of comparison and contrast, seventy-two bovine and eighty-seven human strains.

4. For each bovine strain an exact parallel in cultural characters is exhibited by one or more of the unmodified human strains, the total number of these parallel human strains being thirty-one.

5. The thirty-one human strains which are identical with the seventy-two representative bovine strains belong to sixteen different human viruses. With two exceptions, the primary site of human infection in the case of these viruses was regarded as being some portion of the alimentary tract. The two exceptional instances are derived from a case of lupus and a specimen of mixed sputum.

6. The fifty-six remaining unmodified human strains are different from the seventy-two representative bovine strains. Their difference consists in their possessing a greater degree of cultural luxuriance. With three of these strains, which are placed at the bottom of Grade III., the difference is only slight; with twenty-three, classed in Grade IV., it is greater; and with thirty, classed in Grade V., it is again greater.

7. The human strains, classed in Grades IV.-V., belong to forty-one different viruses. The primary sites of the human infection in these cases are reported as being:—the cervical glands, the mesenteric glands, the bronchial glands, the lungs, the joints, and, in one case, the kidney.

8. To sum up the above considerations, my first three grades exhibit an almost complete parallelism between the bovine and the human strains; the unmodified strains in Grades IV. and V. are all of human origin.

9. The unmodified strains which are enumerated in Grades I.-III. provide in themselves, and independently of any reference to or contrast with the strains enumerated in Grades IV.-V., a complete proof that, judged by the criterion of cultural characters, the bovine bacillus is capable of infecting man. The bovine bacilli, in the left hand columns, are representative of the bovine bacillus as usually met with; and the human bacilli, in the right hand columns, which are identical with these, have, in the majority of cases, produced fatal tuberculosis in the human subject. Moreover, this identity of cultural characters is confirmed by identity of experimental virulence.

Strains Modified by Animal Passage.

1. Five bovine strains, enumerated above, show a decided modification, which is in every case in the direction of increased luxuriance. In two instances this increased luxuriance has brought the strain down into Grade IV., a grade where no unmodified bovine strains occur.

2. If these modified bovine strains be included amongst the representatives of bovine cultural characters, the number of unmodified human strains with cultural characters parallel to the bovine is increased, and, in addition to cases of alimentary tract infection, includes viruses derived from cases where the primary human infection was reported to be situated in the lungs, bronchial glands, wrist joint, and ankle joint.

3. Eleven human strains show a marked modification in cultural characters, the modification being in every case in the direction of diminished luxuriance of growth. These strains belong to three viruses. In one of these the human disease was believed to be primary in the bronchial glands, and in another in the knee-joint. The third case was derived from mixed sputum; this last virus was not subjected to modification by animal passage until it had been shown that the bacilli contained in the original material were not heterogeneous in bacteriological characters; they grew with consistently high luxuriance on culture media, and they exhibited, on animal inoculation, consistently low pathogenic properties.

4. If these modified human strains be regarded from the comparative standpoint, the important fact is established that human viruses, growing with the high degrees of luxuriance exhibited in Grades IV. and V., are capable under favourable animal experiment of acquiring cultural characters identical with those of the unmodified bovine strains classed in Grade I.

5. To sum up, modification by animal passage breaks down the distinction between Grades I.-III. and IV.-V. as representative respectively of viruses culturally identical with, and different from viruses of known bovine origin.

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* Minor differences frequently occur between different strains of the same virus. For example, different strains of the same virus may be classed in different parts of the same grade, or may occur at the bottom of one grade and at the top of the subsequent grade. Those differences have their significance; they illustrate the variability and uncertainty of growth which is characteristic of an organism developing so slowly and capriciously as the tubercle bacillus, both on culture media and in animal tissues. But it would be misleading to attach much importance to these minor differences as instances of modification.

THE RANGE OF VARIATION IN CULTURAL CHARACTERS.

My method of dealing with this subject is as follows. (1) Each virus is taken as being represented by one particular strain, *viz.*, the strain isolated directly from the original material, when available, and, failing that, the strain nearest to the original material. The cultural characters of these "representative" strains are below tabulated according to my previous classification. (2) These strains being taken as the standard, the extent to which other strains diverge from these is exhibited in a chart. (Chart I., end of vol.)

THE CLASSIFICATION OF REPRESENTATIVE STRAINS OF EACH VIRUS.

When each virus is taken as represented by the strain isolated from the original material (when available), or, failing that, by the strain least distantly removed from the original material, the classification works out as follows.*

GRADE I.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original Human Material.
I.	G.P. 14 from original material	Original material	28 C.L.	Cervical glands.
III.	Original material	G.P. 1860 from original material	59 L.B.	Brain.
VI.	Original material	G.P. 1881 from original material	64 M.G.	Meninges.
XII.	G.P. 1159 from original material			
XIII.	Rabbit 116 from original material			
XIX.	Original material			
XXI.	Original material			
XXIII.	Original material			
XXIV.	Original material			
II.	Heifer 100	G.P. 771 from original material	10 B.S.	Mesenteric glands.
XV.	G.P. 1348 from original material	G.P. 1850 from original material	59 L.B.	Mesenteric gland.
XXII.	G.P. 1509 from original material	Original material	59 L.B.	Cervical gland.
XXX.	Original material	Original material	59 L.B.	Lung.
IV.	Original material	G.P. 1835 from original material	65 K.B.	Mesenteric gland.
XVII.	Original material			
XVIII.	Original material			
XXVII.	Original material	G.P. 587 from original material	14 F.S.	Mesenteric glands.
		G.P. 1910 from original material	64 M.G.	Mesenteric glands.

* When cultures have been obtained from different regions of the same human body, each of the strains investigated is included in these tables.

GRADE II.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original Human Material.
VII.	G.P. 1017 from original material	G.P. 1329 from original material	29 M.F.	Cervical glands.
VIII.	Original material			
XIV.	Original material			
XVI.	Original material			
XX.	G.P. 1471 from original material			
XXVI.	Original material			
XXVIII.	Original material			
V.	Heifer 80			
XXV.	Original material	Original material	49 T.C.	Mesenteric glands.
		Calf 213	20 F.L.	Mesenteric glands.
		Original material	31 L.F.	Cervical glands.

GRADE III.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original Human Material.
IX.	Original material	Heifer 239	19 S.W.	Mesenteric glands.
X.	G.P. 1070 from original material	Original material	32 Y.W.	Mesenteric glands.
XI.	Rabbit 66 from original material	Rabbit 166 from original material	32 Y.W.	Bronchial gland.
XXIX.	Original material	G.P. 793 from original material	38 J.M.	Cervical glands.
		G.P. 1482 from original material	53 D.H.	Lupus.
		G.P. 552 from Cow 73	7 C.M.	Mesenteric glands.
		G.P. 690 from Calf 111	2 Sp. A.	Mixed Sputum.
		G.P. 1816 from original material	58 F.G.	Cervical Gland.

GRADE IV.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original Human Material.
		Calf 301	13 A.D.	Bronchial gland and spleen.
		G.P. 999 from Calf 131	18 T.T.	Mesenteric glands.
		G.P. 236 from original material	34 C.U.	Cervical glands.
		G.P. 1571 from original material	36 M.D.	Lung and mesenteric gland.
		G.P. 1567 from original material	52 T.F.	Lung.
		G.P. 1812 from original material	58 F.G.	Lung.
		Original material	8 S.C.	Mesenteric glands.
		G.P. 996 from Calf 183	9 C.T.	Wrist.
		G.P. 1338 from original material	30 E.M.	Mesenteric and bronchial glands.
		G.P. 571 from original material	35 C.B.	Bronchial glands.
		Original material	39 M.B.	Cervical glands.
		G.P. 1447 from original material	42 M.R.	Ankle joint.
		G.P. 1490 from original material	50 H.P.	Lung.
		Original material	55 R.D.	Mesenteric gland.
		Original material	61 E.C.	Mesenteric gland.
		G.P. 1837 from original material	61 E.C.	Brain.
		G.P. 1786 from original material	62 W.M.	Sputum.
		Original material	63 G.R.	Mesenteric gland.
		G.P. 1877 from original material	63 G.R.	Meninges.

GRADE V.

Bovine Viruses.		Human Viruses.		
Number of Virus.	Strain.	Strain.	Designation of Virus.	Original Human Material.
		G.P. 918 from Calf 221	11 E.D.	Elbow joint
		G.P. 577 from original material	12 H.N.	Mesenteric gland
		Calf 311	15 I.W.	Ankle joint
		Calf 157	16 J.H.	Knee joint
		Calf 293	22 F.W.	Lung and bronchial gland
		Calf 365	23 J.P.	Lung
		G.P. 1282 from original material	25 A.T.	Lung
		G.P. 1439 from original material	40 J.G.	Testis
		Original material	44 D.C.	Cervical gland
		Original material	51 H.M.	Lung
		G.P. 1609 from original material	54 C.W.	Bronchial glands
		Original material	55 R.D.	Bronchial gland
		G.P. 1682 from original material	56 F.T.	Lung
		G.P. 1802 from original material	57 B.J.	Mesenteric gland
		G.P. 1807 from original material	57 B.J.	Liver
		Calf 265	17 Sp. B.	Mixed sputum
		G.P. 1326 from original material	26 K.M.	Kidney
		G.P. 1216 from original material	27 B.D.	Cervical gland
		Original material	33 R.T.	Axillary gland
		G.P. 1408 from original material	37 O.J.	Cervical gland
		G.P. 1437 from original material	41 A.S.	Knee joint
		G.P. 1425 from original material	43 F.F.	Knee joint
		G.P. 1137 from original material	45 F.M.	Bronchial gland
		G.P. 1444 from original material	46 H.W.	Knee joint
		G.P. 1430 from original material	47 S.B.	Hip joint
		G.P. 1494 from original material	48 W.P.	Lung
		G.P. 1800 from original material	57 B.J.	Lung

THE RELATION OF CULTURAL CHARACTERS TO EXPERIMENTAL VIRULENCE FOR BOVINES.

The comparative virulence of bovine and human bacilli, in so far as it concerns my department, is dealt with in my Histological Reports. Without entering into finer distinctions and occasional exceptions, it will be sufficient here to deal with a few of the more important facts.

The virulence of the strains in Grades I.–III. is markedly high; that of the strains in Grades IV.–V. is very much lower. In a general way, therefore, there is a parallelism between cultural characters* and virulence, the virulent strains growing less well on artificial media, other than serum, than the slightly virulent. But this parallelism is only roughly true. No gradation in virulence has been found corresponding to the gradation which I have found in cultural characters. For example, many strains in Grade III. have been found as highly virulent as the strains in Grade I.; and many of the strains in Grade IV. have been found as feebly virulent as many of the least virulent in Grade V.

Without entering into the question of intermediate degrees of virulence, and without in any way implying that such intermediate degrees do not exist, it may, then, be stated broadly that whereas there is a gradual and unbroken transition between the cultural characters of the strains which grow least well on artificial media and those which grow most luxuriantly, the transition in the scale of virulence is, on the whole, abrupt. With regard to this difference between culture results and experimental results, some interest attaches to those viruses at the bottom of Grade III. which are of much greater virulence than the viruses at the top of Grade IV., though the difference in the amounts of growth on culture media is only slight. A feature of some significance about the Grade III. cultures is their instability. On broth, for example, the same strain may, on different occasions, produce pellicles of widely different characters. It may form a slightly opaque but thin and uniform pellicle, which refuses to thicken, or a pellicle of moderate but unequal density, or a pellicle which is occasionally as dense as some pellicles produced by Grade V. Another very noticeable character about the broth pellicles formed by Grade III. is that when they grow rapidly they very often become moist and sink. On glycerin-agar and potato there is also some indication of instability amongst these strains. They often begin by growing rapidly and well, as well as Grade IV. strains, but then by about the third week they commence to fall off, as though their original vigour had partially exhausted itself. This lack of cultural stability, therefore, seems to be a feature which distinguishes the better growing virulent strains from less virulent strains.

Apart from the Grade III. strains, there are three interesting cases where there is a marked discrepancy between cultural characters and virulence. The strain of H 16. J.H. through Calf 273 was found by me to be in every way typical of Grade V.; but the bacilli had produced disseminated tuberculosis in Calf 273, after subcutaneous inoculation, and the bacilli transferred from the prescapular gland of this animal into the subcutaneous tissue of Calf 355 produced general tuberculosis in the latter animal. There can be no doubt, therefore, as to the virulence of these bacilli for bovines. But the Calf 273 cultures, ten months after isolation, proved only slightly virulent for bovines in doses of 50 mg. This strain, therefore, provides an interesting counterpart to the feature exhibited by the Grade III. strains. Just as highly virulent strains may be unstable in cultural characters, so strains of high cultural luxuriance may be unstable in their degree of pathogenicity. This same characteristic is brought out by the strain of H 13. A.D. through Calf 301. Culturally, I class this strain in Grade IV.; but the bacilli had produced severe generalised tuberculosis in Calf 301, and, when transferred from the tissues of Calf 301 into Calves 321 and 325, produced a similar condition in the latter animals. But cultures of Calf 301 were afterwards found, in 50 mg. doses, to be of distinctly reduced virulence for bovines, whilst cultures of Calf 321, of the same age (fifteen months) and in the same doses, proved very highly virulent. The cultural characters of the Calf 321 strain I have found to be those of Grade I. This example, therefore, illustrates very clearly both instability of virulence and instability of cultural characters. The third interesting example is the strain of H 17. Sp. B. through Rabbit 181. This strain I class at the bottom of Grade IV. in my culture list. But I find that in the two bovines which were inoculated with 50 mg. of this culture, ten months after isolation, severe generalised tuberculosis was produced. This, then, is another example of the coincidence of marked cultural luxuriance with high pathogenicity.

These observations may be summarised as follows. With the majority of strains there is a general correspondence between cultural characters and virulence, in the sense that those strains which grow poorly on artificial media other than serum are highly virulent, whilst those which grow abundantly on all the artificial media which I have employed are of much lower virulence. But the exceptions to this rule are interesting and important. The relative cultural luxuriance of the Grade III. strains, though most of them are highly virulent, is also an important characteristic. These exceptional cases al exhibit, though in different ways, the characteristic of bacteriological instability. This feature is obviously of high significance in its bearing on the question as to the closeness of relationship between the apparently more stable viruses which diverge from one another in cultural and pathogenic properties.

In order to bring into emphasis the bearing of my results on the general question of the relation of cultural characters to experimental virulence for bovines, I have prepared a series of charts (Charts II.–VIII., see end of volume) which show this relationship in a graphic manner. These charts incorporate the results obtained by the subcutaneous inoculations of 50 mg. of culture into calves, by similar inoculations of 10 mg. of culture, and by subcutaneous inoculations of various doses of tissue emulsions into calves. They also illustrate the modifications observed in the viruses H 13. A.D., H 16. J.H., and H 17. Sp. B.

THE MAIN FACTS ILLUSTRATED BY CHARTS II.–VIII.

Chart II.: 50 mg., Experiments.—(1) Taking the experiments as a whole, there is a wide difference between the severity of the disease caused by different viruses, the viruses which grow less luxuriantly on culture media being very much more virulent than the viruses which grow more luxuriantly. (2) The latter viruses have in no instance produced a severe type of disease. (3) The viruses which grow with less luxuriance have occasionally, but only rarely, produced a slight amount of disease. (4) Virulence for the bovine, therefore, does not diminish *pari passu* with increase of cultural luxuriance, but shows a sudden drop. (5) Viruses of every degree of cultural luxuriance, from the least to the greatest, have produced true tuberculosis in the bovine, and even with the viruses of least virulence this tuberculosis, though not severe, is often disseminate.

Chart III.: 10 mg. Experiments.—(1) Taking the experiments as a whole, the cultures which are virulent for calves in doses of 50 mg. are also virulent in doses of 10 mg. (2) The virulence curve for 10 mg. falls below the virulence curve for 50 mg. (3) The range of variation in the degree of severity of the disease produced by doses of 10 mg. of bacilli identical in cultural characters is much greater than the range with 50 mg. In many instances the animals have survived the 90 days' time limit, and have exhibited *post mortem* a less extensive or a relatively slight amount of disease.

Chart IV.: Subcutaneous Inoculations of Tissue Emulsions.—(1) Viruses which produce severe disease when inoculated in the form of 50 mg. of culture are also capable of producing severe disease when inoculated subcutaneously as tissue emulsions. (2) Viruses not capable of producing severe disease (under the period of observation) when inoculated as 50 mg. of culture have

* Except in the case of strains which had been long resident on artificial media before I commenced my work, my culture classifications have been made and reported to the Commissioners before the experimental determination of virulence was completed.

not produced severe disease when inoculated subcutaneously as tissue emulsions. (3) The tissue emulsion virulence curve (apparently owing to the smallness of the doses with many of the more virulent viruses) falls below the higher portion of the 50 mg. curve and is also below (though occasionally crossing) the 10 mg. curve. (4) Viruses of every degree of cultural luxuriance, from the least to the greatest, have produced true tuberculosis in the bovine, and even with the viruses of least virulence this tuberculosis, though not severe, is often disseminate. (5) Viruses of naturally high virulence have in many instances, generally when the dose has been small or when some other circumstance has favoured the animal's resistance, produced disease of no greater severity than that produced by viruses of naturally low virulence for the bovine.

Charts V.–VII. : Experiments Showing Modification. —The experiments made with the viruses H 13. A.D., H 16. J.H., and H 17. Sp. B. show that a complete transformation has been effected from bacilli with high cultural luxuriance and low virulence for the bovine to bacilli with low cultural luxuriance and high virulence for the bovine.

Chart VIII. : the Combined Results of Charts II.–VII. —This chart shows that, taking the sum total of the observations recorded simply as statements of facts, the characteristics of bacilli possessing high virulence for the bovine are interwoven with the characteristics of bacilli possessing low virulence for the bovine, and that no gap is left between them.

Comments on the Above Facts.

(1) These seven charts (Charts II.–VIII.) exhibit in a graphic manner records of facts. These facts may be compared with the theory that mammalian tubercle bacilli are of two distinct and readily separable types, *viz.*, "bovine" bacilli, which are of high bovine virulence and low cultural luxuriance, and "human" bacilli, which possess little or no virulence for the bovine, but are of high cultural luxuriance. If the facts recorded had conformed to this theory, Charts II.–VIII. would have been very different. They would have all been alike ; they would have simply shown a short transverse line in the upper part of the left half of each chart and another short transverse line close to the bottom of the right half of each chart.

(2) The facts indicated by these charts could only be reconciled with this theory by adopting the following suppositions. (*a*) The range of variation in cultural characters exhibited by my Grades I.–III. must be disregarded as being of no importance ; the range of variation in cultural characters exhibited by my Grades IV.–V. must be disregarded as being of no importance ; the only fact to be considered of importance about cultures must be the difference between the combined characteristics of Grades I.–III., and the combined characteristics of Grades IV.–V. (*b*) The exceptional instances of the production of only slight disease in calves after subcutaneous inoculation with 50 mg. of viruses belonging to Grades I.–III. must be explained away as due to unusual bovine resistance, or some other factor. (*c*) The numerous instances of the production of relatively slight disease by these same viruses when inoculated in doses of 10 mg. must be dismissed on the ground that 10 mg. is an inadequate dose and cannot be relied on as a test of virulence. (*d*) All the tissue emulsion inoculations must be discarded except those where viruses belonging to Grades I.–III. have produced severe disease and those where viruses belonging to Grades IV.–V. have produced no disseminated disease. (*e*) The three viruses which exhibit transformations from one end of the culture and virulence scales to the other must be ignored. (*f*) The fact that the viruses belonging to Grades IV.–V., though of low virulence, produce in the calf a disease which is undoubtedly tuberculosis, must be either ignored or in some manner explained away.

It appears to me that such a method of reconciliation would involve a distortion of the facts.

THE NUTRITIVE PROPERTIES OF VARIOUS LIQUID MEDIA.

I have already dealt with the cultural characters of tubercle bacilli from bovine and human sources when grown on ordinary nutrient broth to which 5 per cent. of glycerin had been added. In this chapter I report my investigations on the nutrient properties of certain other liquid media. The main object underlying this work was to see if, by the use of different substances likely to affect the nutrition of the bacillus, any useful physiological distinctions could be brought out between different viruses or strains.

The cultures selected for examination are representative of each of the five grades into which my cultures fall when classified according to their growth on glycerin-broth, glycerin-agar, and potato.

1. THE INFLUENCE OF VARIOUS SUBSTITUTES FOR GLYCERIN IN BROTH CULTURES.

Method of Investigation.

The basis of the medium used in this investigation was Lemco-peptone broth, containing, per litre, 10 gms. of Lemco, 10 gms. of peptone, and 5 gms. of sodium chloride. Lemco was used owing to its being less variable in its composition than ordinary broths. The medium was coloured with litmus solution and made neutral to this indicator by the addition of sodium carbonate. A portion of the medium so prepared was set apart, without the addition of any other substance, for the purpose of control experiments. To the rest 2·5 per cent. of the substance to be tested was added. The medium was put up in 6 oz. medicine bottles and was inoculated from young glycerin broth cultures. The plugs were sealed with paraffin. In cases where little or no growth took place and there was any reason to suspect that the condition of the material inoculated might be at fault, the experiments were repeated. Weekly observations were made of the amount of growth.

Whilst the main object of the work was to observe the influence of various substances on the growth of the bacilli, it was thought that the addition of litmus might be of some subsidiary use as affording a rough indication of any important change in reaction which might take place during the course of incubation. I do not lay much stress on these observations of changes in reaction, because they are obviously much less exact than careful titrations, and have confined myself to recording any points I have observed which appear to be of some interest. In many cases the litmus became partially or completely decolorised ; the reaction was then tested at the end of the experiment. With some of the sugars it was found difficult to hit off the exact neutral point of the medium, owing to slight decomposition during sterilisation. This difficulty was dealt with in some cases by sterilising fractionally at 75°, and in others by sterilising the sugar separately and afterwards adding it to the medium. Control, non-inoculated bottles were incubated and the colour of these was compared with that of the inoculated bottles.

All cultures were incubated for at least 8 weeks. In some instances there was evidence of commencing or renewed growth during the seventh and eighth weeks ; the cultures were then kept under observation a few weeks longer.

Control Experiments.

These experiments showed that the Lemco-peptone medium, without the addition of any other substance, is not favourable to the growth of the tubercle bacillus. In many cases either the increase in the area of the material inoculated was only very slight or there was no extension at all, the only change being a slight increase in the thickness of the original material. In some instances, with viruses belonging to my Grades I. and II., a greater surface extension took place. In these cases the pellicle formed was always very thin and fragmentary, the yield never being so good as that obtained on glycerin broth with the most poorly growing viruses.

In the majority of cases no change occurred in the colour of the medium, but in two, *viz.*, B V. (Goat 2) and H 2 Sp. A (Calf 111), a slight advance in the alkaline direction was observed.

Details:—

PERIOD OF INCUBATION—EIGHT WEEKS.

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Character of Pellicle.
I.	B III.	Original Material	Very slight	Thin.
„	H 16. J.H.	Calf 423 A	$\frac{2}{3}$	Very thin.
„	H 10. B.S.	G.P. 771	$\frac{1}{3}$	Thin.
„	B I.	Cow 44	$\frac{1}{3}$	Thin.
„	H 14. F.S.	G.P. 587	$\frac{1}{3}$	Very thin.
II.	B VIII.	Original Material	$\frac{1}{4}$	Very thin.
„	B V.	Goat 2	Almost complete	Very thin and fragmentary.
III.	H 7. C.M.	Calf 73	No increase	
	H 2. Sp. A.	Calf 111	Very slight	Moderately thick.
IV.	H 30. E.M.	G.P. 1338	No increase	
V.	H 25. A.T.	G.P. 1282	No increase	
„	H 45. F.M.	G.P. 1137	No increase	

Conclusions :—The more luxuriantly growing viruses (Grades III., IV., and V.) show very decidedly their inability to grow on this medium. The medium is also unfavourable, but to a less degree, for viruses belonging to Grades I. and II.

Glucose.

Glucose proved a very satisfactory substitute for glycerin. All the viruses grew well on the glucose medium, almost as well, if not quite, as on glycerin broth. Perhaps the rapidity of growth was not quite equal to that on glycerin broth, and in some cases there was a delay in the commencement of the growth. H 7. C.M. proved the most troublesome; the first three cultures only showed a small increase and then growth stopped; but the fourth covered the surface with a pellicle characteristic of this virus.

As regards the reaction of the cultures, a slight advance in the alkaline direction was observed with H 16. J.H. (Calf 423 A); the others either showed no well marked deviation from the original reaction or else made a slight advance in the acid direction. With H 2. Sp. A. and H 25. A.T. there was a definite acid reaction in six weeks (confirmed by a second experiment); both these cultures grew luxuriantly, forming a very thick pellicle. It is interesting to note that H 45. F.M. though equally luxuriant in growth, showed no tendency (in two experiments) to move in the acid direction at the end of eight weeks.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Complete	9	Thin; semi-translucent.
„	H 16. J.H.	Calf 423A	„	5	„ „
„	H 10. B.S.	G.P. 771	„	8	Thin; slightly denser in patches.
„	B I.	Cow 44	„	6	Thin; slightly opaque; uniform.
„	H 14. F.S.	G.P. 587	„	5	Thin; semi-translucent; a few denser points.
II.	B VIII.	Original material	„	8	Thin; semi-translucent; rather fragmentary.
„	B V.	Goat 2	„	9	Thin; slightly opaque.
III.	H 7. C.M.	Calf 73	„	$9\frac{1}{2}$	„ „ „
„	H 2. Sp. A.	Calf 111	„	6	Thick; wrinkled; warty.
IV.	H 30. E.M.	G.P. 1338	„	5	Thick; wrinkled.
V.	H 25. A.T.	G.P. 1282	„	6	Thick; warty.
„	H 45. F.M.	G.P. 1137	„	3	Thick; wrinkled; warty.

Conclusions :—The characters of growth of the different viruses on glucose broth are, on the whole, similar to those observed on glycerin broth.

Galactose.

Compared with glucose, galactose proved less favourable and more uncertain. Viruses typical of Grade V. grew well; representatives of Grades I. and II. covered the whole or a considerable part of the surface with material which was generally very thin and fragmentary and inferior in amount to that produced by these viruses on glucose or glycerin broth; viruses which are intermediate in my series yielded poor growths.

With H 25. A.T. the reaction became definitely acid as early as the sixth week, whereas with H 45. F.M. no decided advance in the acid direction was observed, and it was found to be neutral at the end of the ninth week. H 10. B.S., B I. (Cow 44), and B VIII. inclined slightly to the alkaline side. With regard to the rest, I did not note any decided change from the original reaction.

Details:—

Grade (according to previous Classification	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Complete	8	Thin; semi-translucent.
„	H 16. J.H.	Calf 423 A	„	9	Very thin; fragmentary.
„	H 10. B.S.	G.P. 771	$\frac{2}{3}$	5	Thin; semi-translucent.
„	B I.	Cow 44	$\frac{3}{4}$	6	Thin; fragmentary.
„	H 14. F.S.	G.P. 587	Complete	5	Very thin.
II.	B VIII.	Original material	„	9	Thin.
„	B V.	Goat 2	„	7	Thin; fragmentary.
III.	H 7. C.M.	Calf 73	Slight increase	6	Thin; slightly opaque; moist.
„	H 2. Sp. A.	Calf 111	Nearly $\frac{1}{2}$	5	Moderately dense.
IV.	H 30. E.M.	G.P. 1338	$\frac{1}{2}$	8	Rather thin; opaque.
V.	H 25. A.T.	G.P. 1282	Complete	6	Rather dense; tough.
„	H 45. F.M.	G.P. 1137	„	4	Thick; warty.

Conclusions:—Galactose is rather less useful than either glucose or glycerin and does not bring out any new differential features.

Fructose.

Fructose did not prove quite so favourable as glucose, the chief difference noted being that, with the viruses which habitually produce a thick type of pellicle, growth failed to continue until the surface was completely covered.

As the result of sterilising, this medium was slightly acid to litmus to begin with. In the course of incubation no very marked change in reaction was noted, but in some cases there appeared to be a slight advance in the acid direction.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Complete	5	Thin ; uniform.
„	H 16. J.H.	Calf 423A	„	9	„ „
„	H 10. B.S.	G.P. 771	„	5	Thin ; greyish ; uniform.
„	B I.	Cow 44	„	6	Thin ; slightly denser in patches.
„	H 14. F.S.	G.P. 587	„	6	Thin; rather fragmentary.
II.	B VIII.	Original material	„	8	Moderately thin ; somewhat opaque.
„	B V.	Goat 2	„	10	Rather thin ; opaque in patches.
III.	H 7. C.M.	Calf 73	„	6	Thin on the whole, but rather thicker and warty in patches.
„	H 2. Sp. A.	Calf 111	„	8	Moderately dense.
IV.	H 30. E.M.	G.P. 1338	Nearly complete	9	Opaque ; moderately thick ; small islands closely set together
V.	H 25. A.T.	G.P. 1282	Rather more than ½	8	Thick.
„	H 45. F.M.	G.P. 1137	¾	4	Thick.

Conclusions :—No new differential features are brought out by the use of this medium.

Lactose.

The growth was poorer on the whole than with either fructose, galactose or glucose. Many of the viruses belonging to my first three grades managed to cover the whole or the greater part of the surface, but in most of these cases the material formed was extremely thin and fragmentary. H 2. Sp. A. and viruses belonging to the fourth and fifth grades formed a thicker pellicle, but it failed to cover the surface.

In several instances the reaction advanced in the alkaline direction; in no case was there a definite advance in the acid direction.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Almost complete	10	Very thin; fragmentary.
„	H 16. J.H.	Calf 423A	$\frac{2}{3}$	8	Very thin on the whole; a few warty points.
„	H 10. B.S.	G.P. 771	$\frac{3}{4}$	8	Very delicate.
„	B I.	Cow 44	Complete	7	Very thin; uniform.
„	H. 14. F.S.	G.P. 587	„	9	Thin; fragmentary.
II.	B VIII.	Original material	„	8	Not very thin; opaque.
„	B V.	Goat 2	„	7	Very delicate.
III.	H 7 C.M.	Calf 73	„	10	Very thin; fragmentary.
„	H 2. Sp. A.	Calf 111	$\frac{2}{3}$	8	Moderately thick.
IV.	H 30. E.M.	G.P. 1338	$\frac{1}{4}$	8	„ „
V.	H 25. A.T.	G.P. 1282	$\frac{1}{2}$	7	„ „
„	H 45. F.M.	G.P. 1137	$\frac{1}{2}$	4	Warty in patches, but not very thick on the whole.

*Conclusions :—*From these results it appears that lactose is relatively less unfavourable for Grades I.–III. than for Grades IV. and V. But the growth with Grades I.–III. is not, in most cases, as good as that obtained with glycerin or glucose.

Maltose.

The addition of maltose to the medium proved to be of practically no value. In many cases, little, if any, more growth was obtained than in the control experiments.

In several instances there was a slight tendency for the reaction of the medium to advance in the alkaline direction.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Nearly complete	7	Very thin; very fragmentary.
„	H 16. J.H.	Calf 423A	Very slight increase	5	
„	H 10. B.S.	G.P. 771	$\frac{1}{3}$	5	Thin.
„	B I.	Cow 44	$\frac{1}{3}$	7	„
„	H 14 F.S.	G.P. 587	Slight increase	4	
II.	B VIII.	Original material	Very slight increase	9	
„	B V.	Goat 2	No increase	8	
III.	H 7. C.M.	Calf 73	No increase	8	
„	H 2. Sp. A.	Calf 111	Very slight increase	3	
IV.	H 30. E.M.	G.P. 1338	Slight increase	6	
V.	H 25. A.T.	G.P. 1282	$\frac{1}{4}$	8	Thick.
„	H 45. F.M.	G.P. 1137	Slight increase	4	

*Conclusions :—*Maltose appears to have very little influence on the growth of the bacilli.

Saccharose.

A few experiments were tried with saccharose, but as these gave no promise of leading to interesting results, saccharose was abandoned. Slight thickening of the material inoculated was observed, but very little surface extension.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
II.	B VIII.	Original material	Very slight	3	
„	B V.	Goat 2	Slight	2	Some increase in thickness.
III.	H 7. C.M.	Calf 73	„	4	Some increase in thickness.
V.	H 45. F.M.	G.P. 1137	„	4	Thick.

Conclusions :—The above experiments, so far as they go, suggest that saccharose has little or no influence on the growth of the tubercle bacillus.

Raffinose.

The addition of raffinose proved unsatisfactory. Only one culture covered the surface. A culture of this strain, B I. (Calf 122), also covered the surface in a control bottle to which no raffinose or other additional substance was added.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	Complete	6	Thin and uniform on the whole; a few rather thicker patches.
„	H 10. B.S.	G.P. 771	$\frac{1}{4}$	6	Thin.
II.	B. VIII.	Original material	No increase		
„	B V.	Goat 2	„		
III.	H 7. C.M.	Calf 73	„		
V.	H 45. F.M.	G.P. 1137	Slight increase	3	

Conclusions :— There is no satisfactory indication, from these experiments, that raffinose is of value as a help to the growth of the tubercle bacillus.

Mannite.

Very poor results were obtained with this alcohol. With the exception of B I. (Cow 44), the yield was little or no better than in the control experiments, and in some cases less surface extension took place. The reaction of the culture of B I. (Cow 44) definitely advanced in the alkaline direction.

Details :—

PERIOD OF INCUBATION—EIGHT WEEKS.

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Character of Pellicle.
I.	B III.	Original material	Very slight	Thin.
„	H 16. J.H.	Calf 423A	„ „	„
„	H 10. B.S.	G.P. 771	Slight increase	Slight thickening of pellicle.
„	B I.	Cow 44	Complete	Thin.
„	H 14. F.S.	G.P. 587	Very slight	„
II.	B VIII.	Original material	No increase	
„	B V.	Goat 2	„ „	
III.	H 7. C.M.	Calf 73	Slight increase	Very thin.
„	H 2. Sp. A.	Calf 111	„ „	Moderately thick.
IV.	H 30. E.M.	G.P. 1338	No increase	
V.	H 25. A.T.	G.P. 1282	Very slight	Thick.
„	H 45. F.M.	G.P. 1137	Slight increase	„

Conclusions:—It appears improbable that mannite has any nutritive value for the tubercle bacillus.

Dulcite.

After a few experiments the use of this alcohol was abandoned, as there appeared no likelihood that it would lead to useful results.

Details :—

PERIOD OF INCUBATION—EIGHT WEEKS.

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Character of Pellicle.
II.	B VIII.	Original material	Nearly $\frac{1}{4}$	Rather thin.
„	B V.	Goat 2	Very slight	Thin.
III.	H 7. C.M.	Calf 73	„ „	Some thickening of material inoculated.
V.	H 45. F.M.	G.P. 1137	„ „	Thick.

Conclusions :—Dulcite appears to be of no nutritive value.

Erythrite.

Only four experiments are recorded here. Further experiments with erythrite, added to an asparagin medium, are described in a later part of this chapter.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
II.	B VIII.	Original material	$\frac{1}{4}$	6	Thin.
„	B V.	Goat 2	Nearly complete	6	Thin.
III.	H 7. C.M.	Calf 73	Complete	7	Very thin ; fragmentary.
V.	H 45. F.M.	G.P. 1137	Slight increase	5	Moderately thick.

Conclusions :—These results suggested that erythrite might prove to be of some value. I therefore decided to perform further tests with it in an asparagin medium.

Dextrin.

On this medium viruses belonging to Grades I.–III., with the exception of H 2. Sp. A., covered the surface somewhat readily. The pellicle they formed was rarely, if ever, as good as that obtained on glycerin broth, and in many cases was so nearly transparent that it could only be detected by careful examination. The delicate, semi-transparent type of pellicle which many viruses form on glycerin broth generally exhibits fairly numerous small, hemispherical, grey elevations; these were very rarely formed on the dextrin medium, except in the piece of pellicle with which the flask was inoculated. It is also common for delicate glycerin broth cultures to thicken very considerably if kept for longer than two months: this tendency was not exhibited on the dextrin medium; a slight increase in opacity was occasionally observed, but even after three months' incubation there was never any well-marked increase in thickness; after the surface had become covered, growth appeared to cease. With Grades IV. and V. some growth was obtained but the surface never became completely covered; the pellicle formed was not so thin as with the former grades, and therefore the difference in total yield was not so great as would appear from a mere comparison of surface extension.

The reaction generally changed slightly in the alkaline direction. With H 25 A. T., however, there was a slight advance in the acid direction.

Details:—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Complete	6	Very thin; fragmentary.
„	B I.	Calf 122	„	7	Thin; uniform; slightly opaque.
„	H 16. J.H.	Calf 423A	„	4	Very thin; fragmentary.
„	H 28. C.L.	Original material	„	5	Extremely delicate.
„	H 10. B.S.	G.P. 771	„	5	Thin; slightly opaque in patches.
„	B 1	Cow 44	„	6	Thin.
„	H 14. F.S.	G.P. 587	„	7	Very thin.
II.	B VIII.	Original material	„	5	Thin; slightly opaque.
„	B V.	Rabbit 11	„	6	Thin; fragmentary.
„	„	Goat 2	„	7	„ „
III.	H 7. C.M.	Calf 73	„	6	Moderately thin; opaque.
„	H 2. Sp. A.	Calf 111	$\frac{2}{3}$	1	Rather thin; fragmentary.
IV.	H 30. E.M.	G.P. 1338	$\frac{1}{2}$	5	Moderately dense.
V.	H 25. A.T.	G.P. 1282	Nearly $\frac{2}{3}$	10	„ „
„	H 45. F.M.	G.P. 1137	Nearly $\frac{1}{2}$	4	„ „

Conclusions:—Dextrin appears to have some beneficial influence on the medium, though it is decidedly less useful than either glycerin or glucose. This favourable influence is better marked with Grades I.–III. than with Grades IV. and V.

Inulin.

Only four experiments were made with this medium.

Details:—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
II.	B VIII.	Original material	$\frac{2}{3}$	9	Moderately thin; some denser points.
„	B V.	Goat 2	$\frac{2}{3}$	9	Moderately thin; some denser points.
III.	H 7. C.M.	Calf 73	$\frac{3}{4}$	7	Thin.
V.	H 45. F.M.	G.P. 1137	Slight increase	4	Dense.

Conclusions:—Although it does something to improve the medium, inulin does not appear to be a very valuable addition.

Salicin.

Twelve viruses, the same strains as those recorded above in the control experiments, were tested on this medium.

Details:—It is unnecessary to tabulate the results. The bottles were incubated for 8 weeks, but in no case did any increase take place.

Conclusions:—It is evident that salicin has a decidedly inhibitory effect.

Serum as a Substitute for Broth.

I first incubated four viruses on liquid, undiluted bovine serum, viz., B I. (Cow 44), B V. (Goat 2), H 12. H.N. (GP. 577), and H 11. E.D. (Calf 221). They all grew, but very slowly, and never succeeded in covering more than half the surface. As these results were not promising, I then tried a medium consisting of serum diluted with water, together with some additional substance. This method was suggested by the use made by American investigators of a serum-water medium containing some carbohydrate for the purpose of differentiating dysentery bacilli. I used bovine serum which had been stored for some time. This was diluted with water in the proportion of three parts of water to two of serum, and was then heated to 100° for a short time; 2·5 per cent. of some additional substance was then added. With the addition of mannite only slight amounts of growth were obtained in experiments with five viruses, and with the addition of lactose very similar results were obtained in experiments with four viruses. With glucose and with glycerin, however, the growths were good, though not quite so good with glucose as with glycerin. The character of the pellicles formed were the same with glucose as with glycerin.

One object of using a serum-water medium was to see if sufficient acid was formed to coagulate the medium. In no experiment did coagulation occur.

Details of Glycerin serum-water experiments:—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B III.	Original material	Complete	7	Thin; uniform.
„	B I.	Calf 122	„	6	Somewhat thin; slightly opaque.
„	H 14. F.S.	G.P. 587	„	6	Thin.
II.	B V.	Goat 2	„	2	Rather thin; denser in patches.
„	H 31. L.F.	Original material	„	3	Rather thin; denser in patches.
III.	H 2. Sp. A.	Calf 111	$\frac{3}{4}$	8	Thick; warty.
IV.	H 8. S.C.	Calf 361	Complete	4	Moderately thick.
V.	H 25. A.T.	G.P. 1282	$\frac{3}{4}$	8	Thick.
„	H 11. E.D.	Calf 221	$\frac{3}{4}$	6	Thick.

Conclusions:—Serum, even when taken from the same species of animal, is so variable in its nutrient properties as a culture medium for the tubercle bacillus, that the above experiments cannot be regarded as sufficient to justify any general statement as to the value of liquid serum, either diluted or undiluted, for this purpose. They are, however, sufficient to show that glycerin and glucose, when contained in a basis of diluted serum, influence the growth of viruses of different types in much the same way as they do when contained in broth.

II. THE INFLUENCE OF GLYCERIN AND OTHER SUBSTANCES IN A MEDIUM PREPARED FROM KNOWN CHEMICAL COMPOUNDS.

I thought it desirable to control the above results by instituting a series of experiments in which a medium prepared from known chemical ingredients of relatively simple constitution was substituted for broth. For this purpose a medium prepared from the following constituents was taken as the basis :—

Asparagin - - - - -	5 grms.
KH_2PO_4 - - - - -	5 „
Sodium Chloride - - -	5 „
Magnesium Citrate - -	2·5 „
Potassium Sulphate - -	2·5 „
Distilled Water to -	1,000 cc.

Litmus solution was added, and the medium was made neutral to this indicator by the addition of sodium carbonate. A portion of the medium so prepared was set aside for control experiments. To the rest 2·5 per cent. of the substance to be tested was added. The cultures were grown in 6 oz. medicine bottles, sealed with paraffin. All cultures were incubated for at least eight weeks, and weekly observations were made of their progress. The inoculations were made from glycerin broth cultures.

Control Experiments.

Very little growth was obtained. With viruses belonging to Grades I.–III., a portion of the surface was covered with material which was very delicate and often fragmentary. No thickening took place, and the surface never became completely covered.

In some cases the medium was observed to advance slightly in the alkaline direction.

Details :—

PERIOD OF INCUBATION—8 WEEKS.

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Character of Pellicle.
I.	B I.	Calf 122	$\frac{2}{3}$	Very thin ; fragmentary.
„	H 16. J.H.	Calf 423A	$\frac{1}{2}$	Extremely thin ; fragmentary.
„	B I.	Cow 44	$\frac{1}{2}$	Very thin.
„	H 14. F.S.	G.P. 587	$\frac{2}{3}$	„ „
II.	B V.	Goat 2	Slight increase	Thin ; fragmentary.
,	H 20. F.L.	Calf 213	$\frac{1}{3}$	Thin.
„	H 31. L.F.	Original material	$\frac{1}{2}$	Thin ; fragmentary.
III.	H 32. Y.W.	Original material	$\frac{1}{3}$	Extremely thin.
„	H 7. C.M.	Calf 73	No increase	
„	B IX.	Original material	$\frac{3}{4}$	Thin ; fragmentary.
„	H 2. Sp. A.	Calf 111	No increase	
IV.	H 8. S.C.	Calf 361	„ „	
„	H 39. M.B.	Original material	Very slight increase	
„	H 17. Sp. B.	Rabbit 181	No increase	
„	H 42. M.R.	G.P. 1447	„ „	
V.	H 25. A.T.	G.P. 1282	„ „	
„	H 11. E.D.	Calf 221	„ „	

Conclusions :—The behaviour of this medium is similar, on the whole, to that of the Lemco controls. The amount of growth is even less. There can therefore be no difficulty about interpreting the influence of additional substances.

Glycerin.

The glycerinated asparagin medium proved a very useful substitute for ordinary glycerin broth. Comparing the two, it may be said that there is probably a slight difference in favour of glycerin broth. On the asparagin medium some of the difficultly growing viruses produced a very thin, broken pellicle—the kind of material which it is very difficult to transfer from one flask to another, owing to the tenacity with which it adheres to the platinum spatula; and, again, a few of the more easily growing viruses failed to completely cover the surface of this medium. Of course there is always an element of caprice or uncertainty about the growth of the tubercle bacillus on all liquid media, however excellent; but I am inclined to think that these less satisfactory results are rather more frequent on the asparagin than on the broth medium.

Details:—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	Complete	8	Very thin; fragmentary.
”	H 16. J.H.	Calf 423A	”	7	” ”
”	B I.	Cow 44	”	7	Moderately thin; uniform
”	H 14. F.S.	G.P. 587	”	4	Thin.
II.	B V.	Goat 2	”	4	”
”	H 20. F.L.	Calf 213	$\frac{2}{3}$	10	Moderately thin.
”	H 31. L.F.	Original material	Complete	3	Very thin; fragmentary.
III.	H 32. Y.W.	” ”	”	5	Moderately thin; opaque.
”	H 7. C.M.	Calf 73	”	9	Very thin.
”	B IX.	Original material	”	7	Thin; speckled with grey points.
”	H 2. Sp. A.	Calf 111	”	6	Moderately thick.
IV.	H 8. S.C.	Calf 361	”	3	Thick; warty.
”	H 39. M.B.	Original material	$\frac{2}{3}$	7	Thick; wrinkled.
”	H 17. Sp. B.	Rabbit 181	Complete	7	Thick.
”	H 42. M.R.	G.P. 1447	$\frac{2}{3}$	6	Rather thick.
V.	H 25. A.T.	G.P. 1282	Complete	5	Very thick; wrinkled.
”	H 11. E.D.	Calf 221	”	5	Thick; warty.

Conclusions:—A comparison of these results with the results of the control experiments demonstrates very clearly the great value of glycerin. The types of pellicles formed by the different viruses on glycerin asparagin correspond, on the whole, to those formed on glycerin broth.

Glucose.

Glucose was found to be nearly, but not quite, as useful as glycerin. The growth obtained with Grades I.–III. was, on the whole, rather poorer.

The medium never turned acid. The end reaction was always either neutral or slightly alkaline.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	$\frac{3}{4}$	9	Very thin ; fragmentary.
„	H 16. J.H.	Calf 423A	Complete	9	„ „ „
„	B I.	Cow 44	„	8	Thin ; fragmentary.
„	H 14. F.S.	G.P. 587	$\frac{1}{3}$	10	Thin.
II.	B V.	Goat 2	Complete	8	Thin ; fragmentary.
„	H 20. F.L.	Calf 213	„	9	Thin.
„	H 31. L.F.	Original material	Slight increase	4	„
III.	H 32. Y.W.	„ „	Complete	7	Thin ; fragmentary.
„	H 7. C.M.	Calf 73	Slight increase	2	Thin.
„	B IX.	Original material	$\frac{3}{4}$	8	Thin ; fragmentary.
„	H 2. Sp. A.	Calf 111	Complete	3	Rather thick.
IV.	H 8. S.C.	Calf 361	„	4	Very thick ; warty.
„	H 39. M.B.	Original material	Nearly complete	5	Thick ; warty.
„	H 17. Sp. B.	Rabbit 181	Complete	7	„ „
„	H 42. M.R.	G.P. 1447	„	6	Moderately thick.
V.	H 25. A.T.	G.P. 1282	„	4	Very thick.
„	H 11. E.D.	Calf 221	$\frac{3}{4}$	5	Moderately thick.

Conclusions :—The character of the pellicle obtained with glucose corresponds, on the whole, to that obtained with glycerin.

Fructose.

Fructose was found to be much less useful than glucose. Some growth was usually obtained, but the increase was not generally maintained.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	$\frac{1}{2}$	8	Very thin.
„	H 16. J.H.	Calf 423A	Very slight increase	2	
„	B I.	Cow 44	$\frac{1}{4}$	5	Thin ; fragmentary.
„	H 14. F.S.	G.P. 587	Slight increase	4	Thin.
II.	B V.	Goat 2	No increase		
„	H 20. F.L.	Calf 213	Complete	4	Thin ; fragmentary.
„	H 31. L.F.	Original material	$\frac{1}{2}$	4	Very thin.
III.	H 32. Y.W.	„ „	Complete	7	Thin ; speckled with grey points.
„	H 7. C.M.	Calf 73	$\frac{1}{4}$	7	Thin.
„	B IX.	Original material	$\frac{1}{4}$	3	
„	H 2. Sp. A.	Calf 111	$\frac{2}{3}$	6	Thin.
IV.	H 8. S.C.	Calf 361	$\frac{1}{4}$	6	Moderately thick.
„	H 39. M.B.	Original material	$\frac{1}{4}$	7	Thick ; warty.
„	H 17. Sp. B.	Rabbit 181	Slight increase	4	Thick.
„	H 42. M.R.	G.P. 1447	„ „	4	
V.	H 25. A.T.	G.P. 1282	„ „	3	
„	H 11. E.D.	Calf 221	$\frac{2}{3}$	8	Thick.

Conclusions :—No new differential feature is brought out by this medium.

Erythrite.

This alcohol was not found to be of much nutritive value.

The reaction of the medium in some cases advanced slightly in the alkaline direction.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	$\frac{1}{2}$	7	Very thin.
„	H 16. J.H.	Calf 423 A	$\frac{3}{4}$	9	Very thin ; fragmentary.
„	B I.	Cow 44	$\frac{2}{3}$	6	Very thin ; fragmentary.
„	H 14. F.S.	G.P. 587	Very slight increase		
II.	B V.	Goat 2	$\frac{1}{2}$	8	Thin.
„	H 20. F.L.	Calf 213	No increase		
„	H 31. L.F.	Original material	$\frac{1}{2}$	9	Very thin ; fragmentary.
III.	H 32. Y.W.	Original material	Complete	7	Very thin ; some grey points.
„	H 7. C.M.	Calf 73	Very slight increase		
„	B IX.	Original material	Nearly complete	9	Thin ; some grey points.
„	H 2. Sp. A.	Calf 111	$\frac{1}{2}$	6	Thin ; fragmentary.
IV.	H 8. S.C.	Calf 361	Very slight increase		
„	H 39. M.B.	Original material	$\frac{1}{2}$	7	Moderately thin.
„	H 17. Sp. B.	Rabbit 181	Slight increase	7	Thin ; fragmentary.
„	H 42. M.R.	G.P. 1447	Very slight increase		
V.	H 25. A.T.	G.P. 1282	Slight increase	5	Rather thin ; fragmentary.
„	H 11. E.D.	Calf 221	Very slight increase		

Conclusions :—Very little better results were obtained than with the control experiments.

Dextrin.

Dextrin was used with the object of seeing if any further light could be thrown on the apparent tendency in the Lemco experiments, of dextrin to favour the growth of Grades I.–III. but to be of less assistance to Grades IV. and V.

There was a slight tendency for the reaction to move in the alkaline direction.

Details :—

Grade (according to previous Classification).	Virus.	Strain.	Area of Growth compared with Total Surface of Liquid.	Time taken to cover this Area in Weeks.	Character of Pellicle.
I.	B I.	Calf 122	Complete	9	Very thin; slightly denser in patches.
"	H 16. J.H.	Calf 423A	"	9	Very thin.
"	B I.	Cow 44	"	8	Thin: fragmentary.
"	H 14. F.S.	G.P. 587	?	9	Very thin.
II.	B V.	Goat 2	⅔	9	Thin; fragmentary.
"	H 20. F.L.	Calf 213	Complete	3	Very thin; fragmentary.
"	H 31. L.F.	Original material	¾	9	" " "
III.	H 32. Y.W.	" "	Complete	7	Thin; speckled with grey points.
"	H 7. C.M.	Calf 73	½	4	Thin.
"	B. IX.	Original material	Almost covered.	6	"
"	H 2. Sp.A.	Calf 111	⅔	6	Moderately thin; denser in parts.
IV.	H 8. S.C.	Calf 361	Nearly ⅔	7	Moderately thin; denser in parts.
"	H 39. M.B.	Original material	¼	7	Rather dense.
"	H 17. Sp.B.	Rabbit 181	⅓	7	" "
"	H 42. M.R.	G.P. 1147	¼	7	" "
V.	H 25. A.T.	G.P. 1282	Very slight increase		
"	H 11. E.D.	Calf 221	Very slight increase		

Conclusions :—As with the Lemco dextrin cultures, all that can be said is that dextrin appears to have a slight favouring influence on the medium, which is better marked with Grades I.–III. than with Grades IV. and V.

Urea in Place of Asparagin.

Having obtained good results with the glycerin asparagin medium, I determined to try the effect of altering this medium by the substitution of urea for asparagin. Seven viruses were tested on the urea medium. With the slight exception of two, B V. (Goat 2), and H 45. F.M. (G.P. 1137) where the pellicle inoculated increased to two or three times its original area, absolutely no growth was obtained.

SUMMARY AND CONCLUSIONS.

The work recorded in the earlier part of this Report points to the view that all the viruses dealt with belong to the same family, the differences in cultural characters being merely differences in degree of luxuriance on particular media. I had, therefore, no reason for assuming that an application of the "physiological method," which has been found useful in the differentiation of various nearly allied bacteria, would bring out any marked "physiological" differences between various viruses. At the same time I thought it desirable to disregard the indications of my previous work and, on the provisional hypothesis that such physiological differences might exist, to see if I could bring them out.

When an organism grows quickly and vigorously attacks certain carbohydrates or other test substances, with the formation of a well marked and easily recognised change in the reaction of the medium, differentiation from other organisms is easy. But with the tubercle bacillus it is different. Not only does this bacillus grow with great slowness and often with difficulty, but there is no indication, so far as my work goes, that it "attacks" any particular test substance, with the production of readily recognisable decomposition products, in the same way that, for example, many of the micrococci attack and decompose certain of the sugars. It is sometimes assumed that a differentiation of this kind has been found by Theobald Smith, and that his work on glycerin broth cultures shows that the human bacillus forms acid out of that medium, whereas the bovine bacillus does not. If Smith's results* are analysed, it will be found that the final compared with the initial reaction of his medium always showed a diminished acidity and that the advance in the alkaline direction is very variable in amount. If these amounts be tabulated in the order of their numerical values there is nowhere any gap to be found such as would justify the assumption that we are dealing with a difference in physiological properties, and that the bacilli

* *Journal of Medical Research*, Feb. 1905, p. 288.

can be divided into two classes on this basis. The main fact which Smith has established is that when the end reaction shows a marked advance in the alkaline direction the bacillus is probably highly virulent, and when the end reaction shows little or no advance in that direction the bacillus is probably of a low degree of virulence. The total number of human and bovine viruses from which Smith derives his results, in the article to which I refer, is not very large; and an additional difficulty in the way of placing a general interpretation on his conclusions lies in the fact that the weight of the total yield of his broth culture is not stated. Everybody recognises that the average "bovine" bacillus produces on broth a smaller bulk of growth than those human bacilli which are, pathogenically, of less virulence, and culturally, of greater luxuriance. But it is obviously desirable, in order to discriminate between differences in degree and differences in kind, to know whether there is any indication of a direct proportion between final reaction and total yield of growth. It will be noted, in connection with this difficulty, that the "human" bacillus, before it has produced its total yield, moves in its reaction, like the "bovine," in the alkaline direction.

It seems to me, from the experiments I have recorded, that whilst many of the test substances I have used have a marked influence on the growth of the bacilli and whilst, during the growth of the bacilli, slight variations in the acidity or alkalinity of the medium take place, the formation of free acid or alkali is only a subsidiary event in the life history of the bacillus and cannot be made the basis of any fundamental distinction.

With regard to the different test substances used. Of the alcohols glycerin stands out conspicuously as being particularly useful in all the media to which it has been added. No encouraging results were obtained with the other alcohols employed. Attention may be called to the marked absence of all beneficial influence with mannite, which in chemical constitution is closely allied to glycerin and to members of the sixth series of sugars, and with dulcite, which is also closely related to the sugars of the sixth series, particularly to galactose. These results may be taken as an indication of the feebleness of the "attacking" power of the tubercle bacillus. Sugars of the sixth series are clearly helpful to the growth of the bacillus, and there seems to be something in the chemical constitution of glucose which renders it rather more adaptable than either galactose or fructose. Compound sugars are very distinctly less useful, another indication of the feeble ability of the tubercle bacillus to split up chemical compounds. As compound sugars are not readily broken up, it is not likely that glucosides would be; in the case of salicin, the phenol element seems to have a distinctly inhibitory effect. The two starches, dextrin and inulin, seem to be to some small extent useful, particularly the former; owing to the complexity of these bodies, it is difficult to find a chemical explanation for this result.

In answer to the question whether these test media throw any additional light on the differentiation of the viruses under examination, I think the reply is that they serve to bring out various points of difference, but that these differences add very little which is new or important to the differences already established by the use of other media. This conclusion, while in the main negative, may also be taken as an indication that the data on which it is based afford some positive evidence in favour of the view that the cultural differences in the viruses examined are merely differences in degree of luxuriance and do not indicate a more fundamental differentiation into two or more types of bacilli.

THE MICROSCOPIC CHARACTERS OF TUBERCLE BACILLI FROM BOVINE AND HUMAN SOURCES.

I.—BACILLI GROWN ON ARTIFICIAL MEDIA.

Undiluted Serum.

Bacilli grown on horse, bovine, or dog serum are notably uniform in character. They are usually straight and uniformly stained; their average length is about 1μ, sometimes rather less and sometimes rather more. Forms at least as short as $\cdot 75\mu$ are always present, and frequently there are a relatively small number of organisms which measure from $1\cdot 5$ to $2\cdot 5\mu$. There is no important or constant difference between the strains representative of each of my five grades.

Serum is a good, and with minor exceptions, an equally good, medium for all the strains I have examined. This uniformity of microscopic characters appears to be a consequence of the fact that the bacilli are growing under nutritive conditions which are, approximately, equally favourable to each individual strain. I have recorded in tabular form * the measurements of bacilli, grown on horse serum, which are representative of each of my five grades. The measurements have been made with the aid of a micrometer eyepiece, an Ehrlich's stop, and a mechanical stage. Each bacillus when it comes into the centre of the field is measured, provided that there is no indication that its long axis is not lying at right angles to the line of vision. When the bacilli are dealt with systematically in this way it is found that the longer forms, which more readily attract the eye on a casual inspection, are relatively very few in number.

It will be noted from my table that there is no marked difference between bacilli a fortnight old and bacilli which have been growing for a month.

As a control to this table I have selected, eighteen months after the preparation of the films there recorded, fourteen of the same strains, representative of each of my five grades, and again examined the bacilli taken from fifteen days' horse serum growths. Throughout the eighteen months, the strains had been continuously kept up on serum. In only one specimen was any morphological change noted. This was a film from H. 17 Sp. B. (Calf 265). In this case there were many bacilli from 3 to $3\cdot 5\mu$ long, and many were curved.

Glycerinated Serum.

Twelve strains from Grade I. have been examined, six from Grade II., six from Grade III., six from Grade IV., and five from Grade V.

The bacilli from Grade I. were straight and uniformly stained; their length varied from $\cdot 75$ to $2\cdot 5\mu$.

The bacilli from Grade II. were curved and irregularly stained. Their length varied from 1 to $4\cdot 5\mu$, exceptional forms being longer.

The bacilli from Grade III. resembled those from Grade II.

The bacilli from Grade IV. were, on the whole, uniformly stained. Curved forms were more numerous than straight. The length of the bacilli varied from 1 to 3μ.

The bacilli from Grade V. resembled those from Grade IV.

Broth.

Out of 61 strains from Grade I. forty-nine yielded bacilli which were straight, uniformly stained and varied in length from 1μ, or rather less, to about $2\cdot 5\mu$. In one case no growth was obtained. In the remaining eleven strains the bacilli were longer (1 to 4μ) and both curved forms and irregularly stained forms were frequent.

Twenty-two strains from Grade II. have been examined. In nine of these the majority of the bacilli were curved and irregularly stained; their length varied, in each specimen, from about $1\cdot 5$ to 4 or $4\cdot 5\mu$, with occasional longer forms. In the thirteen remaining strains the bacilli were more uniformly stained, shorter (1 to 3 or $3\cdot 5\mu$), and less frequently curved.

Thirty-five strains from Grade III. have been examined. The characters of the bacilli varied in different strains. In about half, the greater number of bacilli were straight and in nearly half they were, for the greater part, uniformly stained; when curved, the bacilli were generally irregularly stained. The length of the bacilli was also variable. The minimum was rarely under 1μ, but the maximum was in some cases not more than 3μ and in others between 4 and 5μ.

In Grade IV. (twenty-seven strains) the bacilli generally ranged between 1 and 3 or $3\cdot 5\mu$ in length, but in five instances they were shorter (1–$2\cdot 5\mu$), and in two they were longer (1–5μ). In sixteen strains curved forms predominated and in seventeen strains uniformly stained forms predominated. In the cases where straight forms were in the majority the bacilli were generally uniformly stained.

In Grade V., thirty strains were examined. The bacilli usually varied in length from 1 to 3 or $3\cdot 5\mu$. In three cases they were rather shorter and in four rather longer. In twenty-two strains uniformly stained forms predominated, and in eighteen strains the greater number of the bacilli were curved.

Glycerin-Agar.

In Grade I. 61 strains were examined. In forty-four of these the bacilli differed only slightly from bacilli grown on serum. They varied in length from $\cdot 75$ to about $2\cdot 5\mu$, and were on the whole straight and uniformly stained, but curved and irregularly stained forms were somewhat less rare than with serum grown bacilli. The remaining seventeen strains diverged from the serum type. In eight, the divergence was marked; curved and irregularly stained forms were numerous, and the length ranged in some cases from 1 to 3 or $3\cdot 5\mu$, and in others from $1\cdot 5$ to 5 or 6μ. In the remaining nine, the divergence from the serum type was less marked.

Out of the twenty-two strains in Grade II., ten were curved and irregularly stained and varied in length from 1 to 4μ or occasionally longer; in eight, curved and irregularly stained forms were rather less frequent and the length was somewhat shorter (1–$3\cdot 5\mu$); and in four, straight forms predominated, irregular staining was less frequent, and the length varied from 1 to $3\cdot 5\mu$.

In Grade III. thirty-five strains were examined. In twenty-four the bacilli were curved and irregularly stained: the length was variable; in some cases it ranged from 1, or less, to 3 or 4μ, and in others from 2, or rather less, to 5 or 6μ. In the remaining eleven strains the bacilli were shorter, and straight and uniformly stained forms were much more frequent.

In Grade IV. the bacilli were, on the whole, similar to those in Grade III. Out of twenty-seven strains I noted a predominance of uniformly stained forms in only seven, and a predominance of straight forms in only three. Amongst the irregularly stained forms, regular, discrete beading was more frequent than with Grade III.

In Grade V., out of thirty strains, curved forms predominated throughout, and in only six instances were uniformly stained forms very numerous. The minimum length, in any one film, was sometimes distinctly above 1μ and, less frequently, rather less than 1μ; the maximum length was never less than 3μ: it was often 4 or $4\cdot 5\mu$, and occasionally from 6 to 8μ.

Potato.

In Grade I. (61 strains), no growth was obtained in thirteen cases. In thirty-five cases the bacilli resembled serum-grown bacilli, with the exception that slightly longer forms and irregularly stained forms were more frequent. In thirteen cases the bacilli showed a tendency to become longer, the longest forms measuring from $3\cdot 5$ to 4μ (rarely 4 to 5μ); the staining was less uniform, and curved forms were more frequent.

In Grade II. (twenty-two strains), no growth was obtained in one case. In ten cases the bacilli resembled slightly modified serum-grown bacilli. In the remaining eleven cases the bacilli varied in length from 1 to 4μ (sometimes 5 or 6μ), and were curved and irregularly stained.

* See pp. 257–261.

In Grade III. (thirty-five strains) the deviation from the serum type was much better marked than in Grade II. In twenty-one cases the bacilli were long, curved, and irregularly stained ; in six cases there was a slight divergence from the serum type, and in eight cases there was a somewhat greater divergence from this type.

In Grade IV., curved bacilli predominated in all but three out of twenty-seven strains. Uniformly stained forms predominated in nine strains. The bacilli were notably long. Forms less than 1μ in length were rare, and the minimum length in a film was generally between 1 and 2μ ; the maximum length was often from $4 \cdot 5$ to 7μ.

In Grade V. (thirty strains) the bacilli in one case presented very little difference from serum grown bacilli. In thirty-three cases the bacilli were curved and irregularly stained and varied in length from a minimum of $\cdot 75$–2μ to a maximum of $3 \cdot 5$–8μ. In the remaining six cases the length was about the same, but curved and irregularly stained forms were rather less frequent. Amongst the irregularly stained forms regular discrete beading was very common.

Summary and Conclusions.

The uniformity in the morphological characters of serum grown bacilli is notable.

Of bacilli grown on other media the most obvious morphological feature is variety. I do not go so far as to say that my morphological data are merely a hopeless and uninteresting chaos. Some sort of explanation may, I think, be provided which, though it does not amount to a precise scientific system, helps to show that the microscopic appearance of a particular bacillus is not altogether a matter of chance.

I preface this explanation by pointing out that in bacteriology it is generally recognised that morphological features, to use a grandiose term for very minute and relatively structureless organisms, are, in minute respects, highly unstable, and therefore are not regarded as of much diagnostic value. I know of no scientific reason why the tubercle bacillus should be regarded as exempt from the application of this general principle.*

In Grade I. the bacilli when transferred from serum to glycerinated serum, broth, glycerin-agar, or potato, show, on the whole, very little microscopic divergence from the serum type. This I regard as evidence of stability. The bacilli grow, though poorly, on these new media, but the media have little power to influence at once their mode of growth.

In Grade II. the bacilli grow on the whole with difficulty, but with less difficulty than Grade I., when transferred to these new media. They are capable of acquiring rather more nutrition out of the media, but this acquisition, being made with difficulty, disturbs them, and the evidence of this disturbance is that they exhibit under the microscope a very much greater divergence from the serum type than Grade I.

In Grade III., where the cultures grow better than in Grade II., but rather capriciously and with lack of stability, there is a still better marked, though somewhat erratic and irregular, divergence from the serum type.

In Grade IV. the bacilli not only diverge from the serum type, they show a tendency to settle down into a different type, the type of bacillus which is much longer than the serum bacillus, shows a well marked curve, and is either uniformly stained or, when stained irregularly, shows a tendency to regular beading. This tendency to settle down may be associated with the fact that these bacilli grow with greater ease than those of Grade III. on most glycerinated media.

In Grade V. this tendency for the organisms to settle down into the type of bacillus which is long, more frequently curved than straight, and either uniformly stained or regularly beaded, is perhaps a little better marked than in Grade IV.

There is, then, on the whole, an indication that, underlying this great diversity of microscopic features, there is a tendency for the bacillus to express by its morphological condition the nutritive influence of the particular medium upon which it is growing.

II.—BACILLI GROWN IN LIVING TISSUES.

I have exhibited in tabular form (pp. 262–273) the microscopic characters of an extensive series of bacilli obtained from human and from animal tissues. It will be seen from this table that bacilli from living tissues are longer and less uniform in appearance than bacilli cultivated on serum. It will also be noted that the bacilli which are less highly pathogenic tend, on the whole, though with notable exceptions, to acquire a rather greater length than the highly virulent bacilli. But though this tendency is recognisable, I think a study of my table will make it sufficiently clear that the virulence of tissue grown bacilli cannot be diagnosed with any certainty from their morphological appearances.

* I am not, of course, referring to the possible occurrence of branched or other peculiar forms, which raise an interesting question of botanical classification. In the films which I have examined branched forms have been very rarely met with, too rarely to attach any special significance to them. It appears, from the work of those who have made a special study of this subject, that various media cause both bovine and human tubercle bacilli to produce branched forms. The following quotation, from an article by Wolbach and Ernst (*Journal of Medical Research*, 1903, Vol. X., p. 329), may be of interest to bacteriologists who attach importance to this subject. "In our own work we have found branched bacilli occasionally in very young bovine cultures (five to eight days) on Dorset's egg medium. We have frequently found them in bouillon cultures, six to twelve weeks old, of the human bacillus. . . . On brain, in young cultures, two to three weeks old, both human and bovine, we have constantly found branched bacilli in large numbers."

MICROSCOPIC CHARACTERS OF BACILLI GROWN ON HORSE SERUM.

Virus.	Strain.	Generation of Culture.	Age of Culture in days.	Lengths of 100 Bacilli.								Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
B I.	G.P. 14 from Original Material	17	14	Length.	1·5μ	1μ	·75μ	·5μ				97	100	·89μ
				Number of bacilli	10	34	50	6						
	,, ,,	17	28	Length.	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ		100	100	·78μ
				Number of bacilli	2	2	2	20	46	28				
	Cow 44 - - - - - -	4	14	Length.	1·5μ	1·25μ	1μ	·75μ	·5μ			99	100	·81μ
				Number of bacilli	3	2	35	36	24					
B II.	Heifer 100 - - - -	7	14	Length.	2μ	1·5μ	1μ	·75μ	·5μ			98	100	·81μ
				Number of bacilli	1	6	21	54	18					
	,,	7	28	Length.	1·5μ	1·25μ	1μ	·75μ	·5μ			97	96	·76μ
				Number of bacilli	4	5	14	45	32					
B III.	Original Material - - -	5	14	Length.	1·5μ	1μ	·75μ	·5μ				100	100	·79μ
				Number of bacilli	5	13	60	22						
	,, - - -	5	28	Length.	1·5μ	1·25μ	1μ	·75μ	·5μ			99	99	·79μ
				Number of bacilli	4	5	20	45	26					
	Monkey 74 - - -	5	14	Length.	2·5μ	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ	94	100	1·05μ
				Number of bacilli	1	8	13	8	32	26	12			

MICROSCOPIC CHARACTERS OF BACILLI GROWN ON HORSE SERUM—*continued.*

Virus.	Strain.	Generation of Culture.	Age of Culture in days.	Lengths of 100 Bacilli.										Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
B IV.	Original Material - - -	8	14	Length.	1·5μ	1μ	·75μ	·5μ						97	100	·86μ
				Number of bacilli	11	32	37	20								
B V.	Goat 2 from Original Material	5	14	Length.	2·5μ	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ			95	100	1·04μ
				Number of bacilli	1	2	17	12	32	29	7					
	Rabbit 11 from Original Material.	5	14	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ				96	96	·99μ
				Number of bacilli	1	5	13	37	31	13						
H 2. Sp. A.	G.P. 690 from Calf 111 - -	7	14	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ	91	96	1·31μ
				Number of bacilli	3	1	2	3	14	19	24	20	14			
H 7. C.M.	Calf 73 - - - - -	8	14	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ				96	99	1·05μ
				Number of bacilli	1	7	18	37	24	13						
H 8. S.C.	Calf 361 - - - - -	2	20	Length.	2μ	1·5μ	1μ	·75μ	·5μ					95	100	·99μ
				Number of bacilli	4	13	50	19	14							
H 9. C.T.	G.P. 996 from Calf 183 - -	6	20	Length.	2μ	1·5μ	1μ	·75μ	·5μ					99	100	·8μ
				Number of bacilli	1	7	24	40	28							

H 10. B.S.	Heifer 231 - - - -	6	14	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ	94	100	·85μ
				Number of bacilli	1	2	4	28	49	16			
	" - - - -	"	28	Length.	1·5μ	1·25μ	1μ	·75μ	·5μ		100	100	·77μ
				Number of bacilli	2	4	16	58	20				
H 11. E.D.	G.P. 918 from Calf 221 - -	4	25	Length.	2μ	1·5μ	1μ	·75μ	·5μ		94	100	1·01μ
				Number of bacilli	5	16	38	33	8				
H 12. H.N.	G.P. 577 from Original Material	7	25	Length.	2μ	1·5μ	1μ	·75μ	·5μ		95	100	·98μ
				Number of bacilli	3	16	35	39	7				
H. 13 A.D.	Calf 301 - - - -	5	20	Length.	2·5μ.	2μ	1·5μ	1μ	·75μ	·5μ	99	100	·91μ
				Number of bacilli	1	3	6	38	38	14			
H 14. F.S.	G.P. 787 from Original Material	9	14	Length.	1·5μ	1μ	·75μ	·5μ			97	100	·78μ
				Number of bacilli	8	22	36	34					
	"	"	28	Length.	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ	98	99	·81μ
				Number of bacilli	3	2	7	14	48	26			
H 15. I.W.	Calf 311 - - - - -	4	19	Length.	2μ	1·5μ	1μ	·75μ	·5μ		97	99	·88μ
				Number of bacilli	2	11	26	46	15				
H 16. J.H.	Calf 157 - - - - -	6	14	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ	97	99	·96μ
				Number of bacilli	2	6	10	29	36	17			

MICROSCOPIC CHARACTERS OF BACILLI GROWN ON HORSE SERUM—*continued.*

Virus.	Strain.	Generation of Culture.	Age of Culture in days.	Lengths of 100 Bacilli.								Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 17. Sp. B.	Calf 265 - - - - -	4	14	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ		90	100	1·08μ
				Number of bacilli	1	9	13	45	26	6				
	„ - - - - - -	„	28	Length.	2·5μ	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ.	95	98	·88μ
				Number of bacilli	3	3	7	4	14	41	28			
H 18. T.T.	G.P. 999 from Calf 131 - -	4	25	Length.	2μ	1·5μ	1μ	·75μ	·5μ			96	100	1·17μ
				Number of bacilli	13	26	33	6	6					
H 19. S.W.	Goat 11 - - - -	5	14	Length.	2μ	1·5μ	1μ	·75μ	·5μ			95	100	·81μ
				Number of bacilli	4	5	23	36	32					
H 20. F.L.	Calf 213 - - - -	5	26	Length.	1·5μ	1μ	·75μ	·5μ				100	100	·76μ
				Number of bacilli	6	13	55	26						
H 22. F.W.	Calf 293 - - - - -	4	14	Length.	2μ	1·5μ	1μ	·75μ	·5μ			98	100	·84μ
				Number of bacilli	1	6	29	51	13					
	„ - - - - - -	„	28	Length.	2μ	1·5μ	1·25μ	1μ	·75μ	·5μ		97	100	·78μ
				Number of bacilli	2	3	3	19	40	33				

H 23. J.P.	Calf 365 - - - -	3	20	Length.	2μ	1·5μ	1μ	·75μ	·5μ			99	98	·63μ
				Number of bacilli	1	2	12	16	69					
H 25. A.T.	G.P. 1282 from original material	4	12	Length.	2μ	1·5μ	1μ	·75μ	·5μ			96	100	·91μ
				Number of bacilli	3	11	31	40	15					
H 27. B.D.	G.P. 1216 from original material	3	14	Length.	1·5μ	1μ	·75μ	·5μ				100	100	·8μ
				Number of bacilli	4	29	45	22						
H 28. C.L.	Original material - - -	4	19	Length.	2μ	1·5μ	1μ	·75μ	·5μ			98	100	·82μ
				Number of bacilli	1	8	22	47	22					
H 29. M.F.	G.P. 1329 from original material	4	8	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ		98	100	·85μ
				Number of bacilli	1	3	6	23	46	21				
H 34. C.U.	G.P. 236 from Original Material.	7	20	Length.	2μ	1·5μ	1μ	·75μ	·5μ			96	99	·9μ
				Number of bacilli	4	9	35	31	21					
H 35. C.B.	G.P. 571 from original material	6	27	Length.	2μ	1·5μ	1μ	·75μ	·5μ			98	99	·89μ
				Number of bacilli	3	8	31	43	15					
H 38. J.M.	G.P. 793 from original material	4	19	Length.	2μ	1·5μ	1μ	·75μ	·5μ			99	100	·82μ
				Number of bacilli	1	1	37	46	15					
H 45. F.M.	G.P. 1137 from original material	5	15	Length.	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ	97	100	1·07μ
				Number of bacilli	2	2	5	9	33	38	11			

MICROSCOPIC CHARACTERS OF BACILLI OUND IN ANIMAL TISSUES.

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.										Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
B I.	Heifer 10	Lung	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			61	61	2·79μ
			Number of bacilli	1	9	12	35	18	19	6					
	Calf 52	Lung	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ					77	88	2·25μ
			Number of bacilli	3	18	24	37	18							
	Cow 64	Milk	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ	90	90	1·46μ
			Number of bacilli	1	1	1	9	18	29	18	16	7			
	Cow 74	Udder	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ				68	98	2·57μ
			Number of bacilli	6	7	25	24	34	4						
	Monkey 2	Lung	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				46	22	3·05μ
			Number of bacilli	2	6	26	41	17	8						
	Monkey 4	Lung	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				55	35	3·13μ
			Number of bacilli	5	16	20	30	18	11						
B II.	G.P.'s 996–999	Various Organs	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ	0·5μ		67	86	1·94μ
			Number of bacilli	5	17	11	26	15	19	6	1				
B III.	Original Material	Bronchial and Mediastinal glands.	Length.	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ				94	95	1·26μ
			Number of bacilli	7	15	21	27	19	11						

			Length.	6·5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			
B III.	Cow 68 - - -	Udder - - -	Number of bacilli	1	2	1	11	16	17	28	24	70	29	2·4μ
			Length.	3μ	2·5μ	2μ	1·5μ	1μ						
B IV.	Original Material - -	Mesenteric gland -	Number of bacilli	1	5	17	36	41				98	86	1·44μ
			Length.	4·5μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				
	Calf 120 - - - -	Prescapular gland -	Number of bacilli	1	1	17	22	27	20	12		76	67	2·82μ
			Length.	3μ	2 5μ	2μ	1·5μ	1μ	0·75μ	0·5μ				
	Calf 138 - - - -	Local Lesion and Prescapular gland.	Number of bacilli	5	7	18	25	18	14	13		83	86	1·41μ
			Length.	4·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ				
	G.P.'s 1101–1102 - - -	Various organs - -	Number of bacilli	1	7	14	28	25	20	5		78	93	1·78μ
			Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ			
B V.	Original Material - -	Mesenteric gland -	Number of bacilli	3	5	12	29	28	16	2	5	78	93	1·75μ
			Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				
H 1. C.M.	Monkey 1 - - - -	Lung - - - -	Number of bacilli	3	4	18	27	29	15	4		30	6	3·32μ
			Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				
	Monkey 3 - - - -	Lung - - - -	Number of bacilli	7	7	20	23	23	16	4		24	46	3·44μ
			Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					
H 2. Sp. A.	Calf 83 - - - -	Lung - - - -	Number of bacilli	4	4	22	23	34	13			67	22	2·41μ

MICROSCOPIC CHARACTERS OF BACILLI FOUND IN ANIMAL TISSUES—*continued.*

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.														Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 2. Sp. A.	Calf 89	Bronchial gland	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ						84	75	2·11μ
			Number of bacilli	2	5	10	26	22	25	5	5								
	G.P. 672	Various organs	Length.	7μ	6·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ		29	21	4·12μ
			Number of bacilli	1	1	10	5	12	12	20	16	13	5	4	1				
H 8. S.C.	Calf 275	Local Lesion	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ				38	34	3·57μ
			Number of bacilli	3	1	6	10	20	19	22	8	9	2						
	Calf 305	Thoracic gland	Length.	7·5μ	7μ	6·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	48	46	3·63μ
			Number of bacilli	1	1	2	5	4	6	12	13	13	8	19	14	2			
	Calf 361	Thoracic gland	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ			50	32	3·08μ
			Number of bacilli	2	2	6	9	14	12	17	8	10	11	9					
	G.P.'s 1228 and 1229	Various organs	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ					54	42	2·72μ
			Number of bacilli	6	12	12	24	18	8	9	4	7							
	G.P.'s 912 to 915	Various organs	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3u	2·5μ	2μ	1·5μ				41	60	2·95μ
			Number of bacilli	1	1	2	6	11	15	18	19	16	11						
H 9. C.T.	Calf 185	Local Lesion	Length.	6·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				48	70	3·49μ
			Number of bacilli	1	3	3	8	10	12	17	15	18	13						

H 9. C.T	Calf 185 - - - -	Prescapular gland -	Length.	6·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ		49	21	3·75μ
			Number of bacilli	1	5	6	9	10	14	21	16	8	7	3				
	Calf 331 - - - -	Prescapular gland -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ	0·5μ	69	74	2·17μ
			Number of bacilli	1	2	4	8	7	11	7	13	19	15	3	10			
	G.P. 1033 - - - -	Various organs - -	Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					45	34	3·01μ
			Number of bacilli	3	6	15	9	27	20	14	6							
H 10. B.S.	Calf 35 - - - -	Prescapular gland -	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ							83	89	2·13μ
			Number of bacilli	2	12	27	34	20	5									
	Calf 113 - - - -	Prescapular gland -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ						85	49	2·1μ
			Number of bacilli	1	3	13	22	31	20	10								
	Calf 199 - - - -	Prescapular gland -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ				75	79	2·14μ
			Number of bacilli	5	3	14	24	18	20	14	1	1						
H 11. E.D.	Calf 137 - - - -	Local lesion -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ					51	7	3·41μ
			Number of bacilli	2	5	11	16	17	27	14	8							
	Calf 309 - - - -	Mesenteric gland -	Length.	6·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ		33	30	3·65μ
			Number of bacilli	2	4	1	12	10	18	15	18	5	9	6				
	G.P.'s 916 to 919 - - -	Various organs - -	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			34	18	3·66μ
			Number of bacilli	2	2	6	20	24	14	10	13	5	4					

MICROSCOPIC CHARACTERS OF BACILLI FOUND IN ANIMAL TISSUES—*continued.*

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.												Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 12. H.N.	Calf 123	Prescapular gland	Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ			65	29	2·66μ
			Number of bacilli	1	1	5	18	19	25	17	12	2					
	Calf 315	Thoracic gland	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			40	89	3·26μ
			Number of bacilli	1	1	11	17	18	27	12	10	3					
	G.P.'s 985 to 988	Various organs	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ		44	94	2·56μ
			Number of bacilli	1	1	2	3	13	21	19	24	10	6				
H 13. A.D.	Calf 119	Prescapular gland	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ				24	4	3·87μ
			Number of bacilli	4	3	8	14	24	23	15	9						
	Calf 301	Thoracic gland	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ					70	78	1·91μ
			Number of bacilli	2	9	11	40	25	11	2							
	G.P.'s 905 to 907	Various organs	Length.	7·5μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	36	63	3·14μ
			Number of bacilli	1	1	1	3	8	15	14	16	19	17	5			
H 14. F.S.	Human	Mesenteric gland	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ						75	42	2·37μ
			Number of bacilli	2	4	16	38	25	15								
	Cow 75	Udder	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			65	25	2·80μ
			Number of bacilli	1	1	7	6	8	28	27	16	6					

H 14. F.S.	Calf 121 - - -	Lung - - -	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ									
			Number of bacilli	6	26	22	42	4							66	74	2·44μ
	Calf 121 - - - -	Prescapular gland -	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ							
			Number of bacilli	1	3	10	17	22	29	18					80	65	2·42μ
	Calf 125 - - - -	Prescapular gland -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ								
			Number of bacilli	2	14	13	26	26	19						71	74	2·41μ
	Heifer 197 - - - -	Prescapular gland -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ							
			Number of bacilli	3	11	22	17	33	10	4					84	63	2·44μ
H 15. I.W.	Calf 193 - - - -	Prescapular gland -	Length.	8μ	7μ	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ			
			Number of bacilli	1	1	1	3	7	17	15	20	15	13	7	29	20	3·7μ
	Calf 367 - - -	Local lesion - -	Length	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					
			Number of bacilli	3	6	17	17	12	16	13	12	4			33	46	3·43μ
	Calf 367 - - - -	Prescapular gland -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				
			Number of bacilli	1	4	9	22	11	15	17	12	7	2		42	37	3·16μ
	Calf 409 - - - -	Liver - - - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ						
			Number of bacilli	1	8	16	19	12	22	14	8				26	12	3·52μ
	G.P. 1053 - - - -	Various organs - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ						
			Number of bacilli	2	3	8	15	29	25	11	7				37	15	3·4μ.

MICROSCOPIC CHARACTERS OF BACILLI FOUND IN ANIMAL TISSUES.—*continued.*

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.												Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 15. I.W.	G.P.'s. 599 to 602 - -	Various organs - -	Length	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ					47	59	3·36μ
			Number of bacilli	1	15	22	16	18	19	9							
	G.P.'s 967 to 970 - -	Various organs - -	Length	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				47	99	2·71μ
			Number of bacilli	1	13	13	17	22	26	6	2						
H 16. J.H.	Calf 157 - - - -	Prescapular gland -	Length	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	43	24	3·07μ
			Number of bacilli	2	2	6	9	9	14	13	16	15	12	2			
	Calf 187 - - - -	Local lesion and gland	Length	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ			44	85	2·9μ
			Number of bacilli	5	7	8	8	24	23	15	8	2					
	Calf 273 - - - -	Prescapular gland -	Length	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ				79	85	1·91μ
			Number of bacilli	1	2	10	24	19	20	17	7						
	Calf 355 - - - -	Prescapular gland -	Length	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	0·75μ	0·5μ		77	62	1·9μ
			Number of bacilli	2	1	2	11	18	22	18	13	7	6				
	Rabbit 66 - - - -	Various organs - -	Length	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					66	67	2·57μ
			Number of bacilli	2	4	9	23	25	27	10							
H 17. Sp. B.	Calf 391 - - - -	Local lesion and prescapular gland.	Length	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ				32	50	3·2μ
			Number of bacilli	3	10	16	12	29	16	12	2						

H 17. Sp. B.	G.P. 1023 - - - -	Various organs - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5	2μ	1·5μ			41	17	2·95μ
			Number of bacilli	2	1	4	14	18	14	21	19	7					
	G.P.'s 1132 and 1133 - -	Various organs - -	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ		41	33	3·4μ
			Number of bacilli	2	2	10	9	11	12	27	15	8	4				
H 18. T.T.	Human - - - -	Mesenteric gland -	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ			61	49	2·56μ
			Number of bacilli	7	8	14	16	13	17	10	10	5					
	Calf 165 - - - -	Prescapular gland -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				47	33	3·11μ
			Number of bacilli	2	2	11	8	16	17	28	16						
	Calf 405 - - - -	Local lesion and Prescapular gland.	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	40	69	3·67μ
			Number of bacilli	2	2	9	16	23	10	18	9	5	4	1			
	G.P. 1173 - - - -	Various organs - -	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ			35	4	3·52μ
			Number of bacilli	1	2	4	13	24	14	20	16	6					
H 19. S.W.	Human - - - -	Mesenteric gland -	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ					78	81	1·64μ
			Number of bacilli	1	4	16	21	25	19	14							
	Calf 159 - - - -	Prescapular gland -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ					96	67	1·91μ
			Number of bacilli	1	1	7	19	29	29	14							
	Calf 179 - - - -	Prescapular gland -	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ					83	93	1·99μ
			Number of bacilli	5	3	19	42	21	7	3							

MICROSCOPIC CHARACTERS OF BACILLI FOUND IN ANIMAL TISSUES.—*continued.*

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.											Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 19. S.W.	Calf 271	Prescapular gland	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				61	35	2·42μ
			Number of bacilli	1	11	22	25	24	13	4						
H 20. F.L.	Human	Mesenteric gland	Length.	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ				83	95	1·52μ
			Number of bacilli	3	10	20	29	27	8	3						
	Calf 213	Prescapular gland	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ				69	69	1·99μ
			Number of bacilli	5	12	17	26	23	15	2						
	Heifer 253	Prescapular gland	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				50	82	2·35μ
			Number of bacilli	1	5	24	21	33	14	2						
	G.P.'s 1153 and 1154	Various organs	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ		83	92	1·75μ
			Number of bacilli	3	5	8	7	22	20	18	8	9				
H 21. G.B.	G.P.'s 1236 and 1237	Various organs	Length.	5·5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ		43	77	3·02μ
			Number of bacilli	1	8	17	9	27	17	13	6	2				
H 22. F.W.	Human	Lung	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	44	50	2·89μ
			Number of bacilli	2	1	7	10	14	19	16	21	7	3			
	Calf 399	Thoracic gland	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	24	77	3·55μ
			Number of bacilli	1	1	8	13	23	13	19	16	4	2			

H 22. F.W.	G.P. 1200 - - - -	Various organs - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ			
			Number of bacilli	1	3	8	10	22	18	15	14	7	2	51	57	3·05μ
H 23. J.P.	Human - - -	Lung - - - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ				
			Number of bacilli	2	4	9	15	12	21	9	21	7		43	43	3·11μ
	Calf 345 - - - -	Prescapular gland -	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ					
			Number of bacilli	4	8	9	21	17	19	16	3			48	46	2·61μ
	Calf 365 - - - -	Prescapular gland -	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ				
			Number of bacilli	2	9	6	30	22	14	10	5	22		59	33	2·6μ
H 24. A.B.	Human - - - -	Mesenteric gland -	Length.	3μ	2·5μ	2μ	1·5μ	1μ	·75μ	·5μ						
			Number of bacilli	3	8	12	16	22	19	2				81	84	1·23μ
H 25. A.T.	Human - - - -	Lung - - - -	Length.	5·5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ	·75μ			
			Number of bacilli	1	9	9	8	7	10	22	13	12	9	60	58	2·38μ
	Bull 551 - - - -	Thoracic gland - -	Length.	6μ	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ				
			Number of bacilli	6	4	10	12	22	13	18	7	8		28	76	3·83μ
	G.P. 1282 - - - -	Various organs - -	Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					
			Number of bacilli	3	10	16	14	32	12	12	1			39	58	3·24μ
H 26. K.M.	Human - . - -	Kidney - - -	Length.	5·5μ	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ				
			Number of bacilli	2	9	14	22	17	16	8	9	3		33	43	3·57μ

MICROSCOPIC CHARACTERS OF BACILLI FOUND IN ANIMAL TISSUES—*continued.*

Virus.	Animal.	Nature of Tissue.	Lengths of 100 Bacilli.									Percentage of Straight Bacilli.	Percentage of Uniformly Stained Bacilli.	Average Length.
H 27. B.D.	G.P.'s 1216 to 1219 - -	Various organs - -	Length.	5μ	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ			
			Number of bacilli	2	6	12	13	22	19	17	9	45	81	2·91μ
H 28. C.L.	Calf 515 - - - -	Prepectoral and Cervical glands	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ				
			Number of bacilli	6	11	15	20	19	21	8		63	37	2·34μ
	Rabbit 87 - - - -	Various organs - -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					
			Number of bacilli	4	2	13	15	39	27			61	95	2·18μ
	G.P.'s 1270 and 1271 - -	Various organs - -	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ					
			Number of bacilli	9	16	19	36	16	4			60	99	2·27μ
H 29. M.F.	Calf 479 - - - -	Prescapular, Prepectoral and Cervical glands.	Length.	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ					
			Number of bacilli	3	11	12	30	30	14			83		1·92μ
	G.P.'s 1328 and 1329 - -	Various organs - -	Length.	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ					
			Number of bacilli	1	13	23	21	26	16			48	16	2·45μ
H 30. E.M.	G.P.'s 1336 and 1339 - -	Various organs - -	Length.	4·5μ	4μ	3·5μ	3μ	2·5μ	2μ	1·5μ	1μ			
			Number of bacilli	3	12	13	18	16	24	12	2	50	37	2·69μ

H 32 Y.W.	Human - - - -	Lung - - -	Length.	4μ	$3 \cdot 5\mu$	3μ	$2 \cdot 5\mu$	2μ	$1 \cdot 5\mu$				
			Number of bacilli	6	6	17	21	33	17		57	76	$2 \cdot 4\mu$
	Human - - - -	Mesenteric gland -	Length.	4μ	$3 \cdot 5\mu$	3μ	$2 \cdot 5\mu$	2μ	$1 \cdot 5\mu$				
			Number of bacilli	8	8	18	24	26	16		63	72	$2 \cdot 5\mu$
H 36. M.D.	Human - - - -	Bronchial gland -	Length.	4μ	$3 \cdot 5\mu$	3μ	$2 \cdot 5\mu$	2μ	$1 \cdot 5\mu$	1μ			
			Number of bacilli	2	9	19	21	29	13	7	54	79	$2 \cdot 32\mu$
H 8. S.C. + H 10. B.S.	Calf 689 - - - -	Lung - - - -	Length.	$4 \cdot 5\mu$	4μ	$3 \cdot 5\mu$	3μ	$2 \cdot 5\mu$	2μ	$1 \cdot 5\mu$			
			Number of bacilli	3	7	11	30	20	24	5	54	73	$2 \cdot 75\mu$

CONCLUDING REMARKS.

I have examined in minute detail the cultural characters of thirty viruses derived from spontaneously infected bovines. These viruses are recognised as representing the types of tubercle bacilli commonly met with in the bovine. By identical methods and on identical media I have investigated fifty-seven viruses of human origin. Of these latter sixteen correspond in every respect with the characters of the bovine cultures. This identity is a complete identity of detailed observations, and is not dependent on any theoretical interpretation of these observations. The bovine strains exhibit cultural differences from one another, and so do the human strains belonging to these sixteen human viruses; but whether these differences be regarded as great or small, important or unimportant, the fact remains that for every feature exhibited by a bovine strain an identical feature has been found in one or other of the human strains referred to, and *vice versa*. It is, therefore, established that, judged by the criterion of bacteriological culture, the bovine tubercle bacilli and these human tubercle bacilli are identical organisms.

I have found that forty-one human viruses exhibit, either consistently or at least prior to experimental modification, differences from the bovine viruses. These differences consist in every case of a greater cultural luxuriance on media other than pure serum. They are differences of degree and not of kind. No medium has been found which allows the growth of these human bacilli whilst it prevents the growth of the bovine bacilli, nor, conversely, has any medium been found which distinctly favours the growth of the bovine bacilli whilst it hinders the growth of the human bacilli. But whilst differences of degree only, these differences are definite, and, therefore, apart from any question of the high or low degree of their magnitude or importance, they must be regarded as objective differences, based on detailed comparisons.

Belonging to certain viruses, both bovine and human, strains have been found, after experimental animal passage, where the cultural characters diverge to a marked extent from the degree of luxuriance originally exhibited by the viruses to which they belong. This divergence is so great that it overlaps the separation between the cultural characters common to all non-modified bovine strains and the characters common to those human strains which differ, in respect of luxuriance, from the non-modified bovine. In view of this overlapping, it is impossible to assert that, because a strain exhibits certain definite cultural characters, the bovine or non-bovine origin of that strain can, *ipso facto* and without reference to the possibility of modification having taken place in the living tissues, be either positively affirmed or categorically excluded.

Divergent Opinions on Bacteriological Characteristics.

The conclusions I have formulated are not in complete accordance with the opinions expressed by some of the leading authorities abroad.

Before commenting on any of these differences of opinion, I wish to acknowledge the great advantage I have derived from a study of work already published on the cultural characteristics of bovine and human tubercle bacilli. These publications are so numerous that it is impossible for me to specify them in detail, but I feel I ought to make particular mention of the work published by Kossel and his colleagues in Berlin.

Kossel describes certain definite cultural and morphological characters as being typical of tubercle bacilli of bovine origin, and contrasts these with other characters which he finds typical of the "human type" of bacillus. It is possible for me to pick out of the material I have examined bovine viruses which are in complete accordance, in essential respects, with Kossel's descriptions of the *Typus Bovinus*; I can also find numerous human viruses which agree, in their main cultural features, with his *Typus Humanus*. My difficulty is that the viruses so selected would not constitute the whole of my material. Quite apart from certain human viruses which Kossel would probably recognise as exhibiting the cultural characters of his *Typus Bovinus*, I find that some of my strains of bovine origin and some of my strains of human origin are not in accordance with Kossel's account either of the *Typus Bovinus* or of the *Typus Humanus*. I feel unable to disregard these strains, nor can I admit that the characters they exhibit are unimportant. My conclusions must be based on my results as a whole, without applying any principle of selection or exclusion. And the only conclusion at which I can arrive is that a hard and fast demarcation between a *Typus Bovinus* and a *Typus Humanus* would not be compatible with all the facts which have come under my observation. The only element of truth underlying this demarcation is, in my opinion, that with mammalian tubercle bacilli, as with many other micro-organisms, the strains of greatest virulence adapt themselves less readily to artificial culture than do strains of less virulence.

Arthur Eastwood.

VIRUSES INVESTIGATED.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
B I.	Baboons :—	Original material.
	2	Baboon 8.
	6	Baboon 64.
	8	Baboon 66.
		Calf 122.
	Bovines :—	Cow 44.
	8	Monkey 80.
	10	Monkey 82.
	18	Pig 8.
	26	Pig 96.
	30	Pig 98.
	40	
	44	
	48	
	52	
	64	
	74	
	102	
	122	
	124	
	126	
	128	
	132	
	146	
	154	
	156	
	222	
	230	
	Cat :—	
	26	
	Monkeys :—	
	2	
	4	
	10	
	18	
	62	
	70	
	76	
	Orang :—	
	4	
	Pigs :—	
	2	
	8	
	18	
	20	
	22	
	24	
	26	
	30	
	34	

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
B I.—*cont.*	Pigs :—	
	36	
	38	
	40	
	42	
	Rabbits :—	
	83	
	113	
	225	
	Rat :—	
	47	
B II.	Bovines :—	
	4	Calf 134.
	14	G.P. series.
	50	Heifer 100.
	66	
	114	
	212	
	226	
	500	
	Goat :—	
	10	
	Pig :—	
	2	
	Rabbit :—	
	152	
B III.	Bovines :—	Original material.
	68	Monkey 74.
	108	Mouse 10.
	116	
	210	
	214	
	248	
	Cats :—	
	32	
	34	
	36	
	Rabbits :—	
	(A series)	
	87	
B IV.	Baboons :—	Original material.
	20	Baboon 22.
	46	Baboon 62.
	62	Cat 12.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
B IV.—*cont.*	Bovines :—	Chimpanzee 4.
	140	Chimpanzee 6.
	142	Chimpanzee 8.
	172	Chimpanzee 10.
	188	Chimpanzee 16.
	218	Cow 172.
	292	Dog 18.
	Chimpanzees :—	Dog 92.
	2	Dog 116.
	4	Rat 42.
	6	
	8	
	10	
	16	
	Dogs :—	
	26	
	74	
	92	
	Goat :—	
	20	
	Pigs :—	
	58	
	80	
	92	
	94	
	142	
	144	
	146	
	148	
	150	
	Rabbits :—	
	89	
	239	
	Rat :—	
	42	
B V.	Bovines :—	Dog 80.
	88	Goat 2.
	144	Heifer 80.
	224	Rabbit 11.
	246	
	352	
	354	
	Goats :—	
	2	
	23	
	Rabbit :—	
	145	
B VI.	Bovines :—	Original material.
	216	Calf 240.
	240	
	330	
B VI.—*cont.*	Rat :—	
	37	
B VII.	Bovines :—	Original material.
	236	
	238	
	Rat :—	
	48	
B VIII.	Bovines :—	Original material.
	254	
	256	
	Rat :—	
	61	
B IX.	Bovines :—	Original material.
	242	Baboon 68.
	244	Cat 52.
	320	Chimpanzee 14.
	380	Dog 50.
	Cats :—	Dog 60.
	42	Dog 84.
	48	Goat 32.
	50	Hedgehog 22.
	52	Lemur 10.
	Chimpanzees :—	Mongoose 14.
	14	Monkey 116.
	18	Pig 108.
	Dogs :—	Rabbit 347.
	58	
	114	
	Lemurs :—	
	2	
	10	
	12	
	Mandrill :—	
	54	
	Mangabeys :—	
	2	
	4	
	Monkeys :—	
	108	
	110	
	114	
	116	
	130	
	Pigs :—	
	108	
	114	
	Rabbit :—	
	535	

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.	Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
B X.	Bovine :— 260	Original material.	**B XIX.**	Bovine :— 288	Original material.
B XI.	Bovines :— 232 234 Rabbits :— 163 563 564 565 567 570	Rabbit 66.	**B XX.**		Original material.
			B XXI.		Original material.
			B XXII.		Original material. Dog 72.
			B XXIII.	Bovines :— 344 346	Original material.
B XII.	Bovine :— 276 Rat :— 55	Original material. Rabbit 98.	**B XXIV.**	Bovines :— 340 342	Original material. (Lung.) Original material. (Spleen).
B XIII.	Bovine :— 272	Rabbit 116.	**B XXV.**		Original material.
			B XXVI.	Monkey :— 142	Original material.
B XIV.	Bovines :— 250 252	Original material.	**B XXVII.**		Original material.
B XV.	Bovines :— 270 278	Original material.	**B XXVIII.**	Cats :— 76 84	Original material.
B XVI.	Bovines :— 294 296 Rat :— 73	Original material.	**B XXIX.**		Original material.
			B XXX.		Original material.
			H 1. C.M.	Bovines :— 3 21 Monkeys :— 1 3 7 9	
B XVII.	Bovine :— 258	Original material.			
B XVIII.	Bovines :— 284 360 362 364 366 Cat :— 66	Original material.	**H 2. Sp. A.**	Bovines :— 11 63 79 83	Calf 93. Calf 111.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 2. Sp. A.—*cont.*	Bovines :—	
	85	
	89	
	93	
	153	
	569	
	575	
	883	
	889	
H 7. C.M.	Bovines :—	Calf 103.
	5	Cow 73.
	73	
	101	
	103	
	105	
	109	
	141	
	525	
	563	
	773	
	Rabbits :—	
	(A series).	
	201	
H 8. S.C.	Bovines :—	Original material.
	267	Calf 361.
	305	
	361	
	657	
	Dog :—	
	11	
	Goat :—	
	19	
	Monkey :—	
	17	
H 9. C.T.	Bovine :—	Calf 183.
	185	
H 10. B.S.	Bovines :—	Original material.
	81	Goat 3.
	191	Heifer 231.
	231	
	249	
	295	
	387	
	667	
	967	
	969	
	Goat :—	
	3	
H 10. B.S.—*cont.*	Monkeys :—	
	53	
	55	
	59	
	Rabbits :—	
	296	
	298	
H 11. E.D.		Calf 221.
H 12. H.N.	Bovines :—	Original material
	135	Rabbit 640.
	319	
	619	
	Rabbit :—	
	72	
H 13. A.D.	Bovines :—	Calf 301.
	301	Calf 321.
	325	Rat 15.
	701	
	825	
	Rabbit :—	
	38	
	Rat :—	
	15	
H 14. F.S.	Bovines :—	Original material.
	75	Calf 125.
	121	Calf 327.
	125	Calf 895.
	197	
	243	
	545	
	671	
	893	
	895	
	Goat :—	
	5	
	Rabbit :—	
	278	
H 15. I.W.		Calf 311.
H 16. J.H.	Bovines :—	Calf 157.
	245	Calf 273.
	273	Calf 337.
	281	Calf 355.
	355	Calf 423A.
	541	Calf 559.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 16. J.H.—*cont.*	Bovines :—	
	559	
	711	
	779	
	789	
	Rats :—	
	28	
	30	
	36	
H 17. Sp. B.	Bovines :—	Calf 265.
	529	Calf 339.
	555	Calf 553.
	607	Calf 555.
	685	Calf 571.
	739	Rabbit 181.
	897	
	901	
	911	
	919	
	Goats :—	
	1	
	39	
	Rabbit :—	
	231	
	Rat :—	
	34	
H 18. T.T.	Bovines :—	Calf 131.
	165	Calf 405.
	405	
H 19. S.W.	Bovines :—	Goat 11.
	159	Heifer 239.
	233	
	239	
	271	
	577	
	597	
	885	
	913	
	921	
	Rabbit :—	
	235	
	Rats :—	
	13	
	14	
H 20. F.L.	Bovines :—	Calf 213.
	547	
	561	
H 20. F.L.—*cont.*	Bovines :—	
	867	
	879	
	Rat :—	
	33	
H 22. F.W.	Bovines :—	Calf 293.
	399	
	749	
H 23. J.P.	Bovines :—	Calf 365.
	365	
	441	
	625	
	Rabbit :—	
	241	
H 25. A.T.	Bovines :—	Original material.
	551	Chimpanzee 1.
	831	Chimpanzee 3.
	859	Dog 1.
	Chimpanzees :—	
	1	
	3	
	Dogs :—	
	1	
	3	
H 26. K.M.	Bovines :—	Original material.
	869	
	871	
	873	
	881	
H 27. B.D.	Rabbit :—	Original material.
	304	
H 28. C.L.	Bovines :—	Original material.
	143	Monkey 63.
	489	
	813	
	819	
	863	
	875	
	877	
	Goat :—	
	1	
	Monkeys :—	
	63	
	65	

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 28. C.L.—*cont.*	Rats :— 24 27 39	
H. 29. M.F.	Bovines :— 251 477 693 697 835 Rabbit :— 274 Rat :— 35	Original material.
H 30. E.M.	Bovines :— 593 599 Rabbits :— 212 215	Original material
H 31. L.F.	Bovines :— 519 521 939 947 Goat :— 35	Original material.
H 32. Y.W.	Bovines :— 587 589 837 849 851 855 Goats : – 1 7 19	Human bronchial gland. Human mesenteric gland.
H 33. R.T.	Bovine :— 595 Rabbits :— 217 219	Original material.
H 34. C.U.		Original material.
H 35. C.B.	Bovine :— 605	Original material.
H 36. M.D.		Original material.
H 37. O.J.	Bovine :— 627 Rabbits :— 238 240	Original material.
H 38. J.M.	Bovines :— 643 827 845 Rabbit :— 250	Original material.
H 39. M.B.		Original material.
H 40. J.G.		Original material.
H 41. A.S.	Rabbit :— 320	Original material.
H 42. M.R.		Original material.
H 43. F.F.	Rabbit :— 328	Original material.
H 44. D.C.	Rabbit :— 346	Original material.
H 45. F.M.		Original material.
H 46. H.W.	Bovine :— 767	Original material.
H 47. S.B.	Rats :— 37 38	Original material.
H 48. W.P.		Original material.
H 49. T.C.	Bovines :— 787 929	Original material. Calf 787. Calf 797.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 49. T.C.—*cont.*	Bovines :— 931 933 945 949 957 959	
H 50. P.H.	Rat:— 40	Original material.
H 51. H.M.		Original material.
H 52. T.F.		Original material.
H 53. D.H.	Bovines :— 905 975 993	Original material. Calf 905.
H 54. C.W.	Rat :— 45	Original material.
H 55. R.D.		Human bronchial gland. Human mesenteric gland.
H 56. F.T.	Baboons :— 1 3 Pigs :— 21 23	Original material.

Designation of Viruses.	Animals Examined Histologically.	Origins of Cultures Examined.
H 57. B.J.		Human liver. Human lung. Human mesenteric gland.
H 58. F.G.		Human cervical gland. Human lung.
H 59. L.B.	Bovines :— 965 987 1037 1057 1063 1077	Human brain. Human cervical gland. Human lung. Human mesenteric gland.
H 61. E.C.	Monkeys :— 73 75	Human brain. Human mesenteric gland.
H 62. W.M.		Original material.
H 63. G.R.	Dog :— 15	Human meninges. Human mesenteric gland.
H 64. M.G.	Bovine :— 1091	Human meninges. Human mesenteric gland.
H 65. K.B.		Original material.

ANIMALS HISTOLOGICALLY INVESTIGATED.

TABULATED ACCORDING TO THE METHOD OF INFECTION.

(a) ANTHROPOID APES AND MONKEYS.

Method of Infection.	Designation of Animal.	Pages.
Feeding	Baboons :—	
	1	121
	2	105
	6	105
	8	105
	20	109
	62	110
	Chimpanzees :—	
	2	110
	3	120
	6	111
	8	111
	10	111
	14	112
	16	112
	18	112
	Lemur :—	
	2	113
	Mandrill :—	
	54	114
	Mangabeys :—	
	2	114
	4	114
	Monkeys :—	
	53	118
	55	119
	59	119
	62	108
	63	120
	65	121
	70	109
	76	109
	108	115
Feeding—*cont.*	Monkeys :—	
	110	115
	130	116
	Orang :—	
	4	109
Intravenous	Monkeys :—	
	4	107
	18	108
Subcutaneous	Baboons :—	
	3	121
	46	110
	Chimpanzees :—	
	1	120
	4	110
	Lemurs :—	
	10	113
	12	113
	Monkeys :—	
	1	116
	2	106
	3	117
	7	117
	9	118
	10	107
	17	118
	73	122
	75	122
	114	115
	116	115
	142	116

(b) **BOVINES.**

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding.	Calves :—		Intraperitoneal	Calves :—	
	21	87		93	50
	50	84		109	52, 64, 76, 89
	116	85			
	146	84	Intravenous	Calves :—	
	154	84		89	49, 87
	156	84		101	51, 75, 88
	188	86		305	52, 64, 76, 90
	380	48, 63, 75, 86		319	53, 66, 77
				361	52, 65, 76, 91
	Heifer :—			399	58, 70, 80, 94
	11	87		441	59, 70
				529	56, 68, 79, 93
				551	59, 70, 80, 95
Intramammary	Cows :—			607	57
	3	39, 86		685	57, 93
	4	37, 84		749	58, 70, 80, 94
	18	35, 82		831	59, 71, 80, 95
	40	35, 44, 82		859	59, 71, 80
	44	36		Heifers :—	
	64	36		8	43
	68	38, 47		10	43
	73	39, 88		26	43
	74	36, 46, 83			
	75	40	Subcutaneous.	All bovines not infected by other methods.	
	143	42			
	172	39, 47			
	295	40	Subperitoneal	Calf :—	
	500	37, 85		85	49

(c) **CATS.**

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding	Cats :—				
	42	142	Subcutaneous	Cats :—	
	48	142		26	141
	50	142		32	141
	52	142		66	143
				76	143
				84	143
Intraperitoneal	Cats :—				
	34	141			
	36	141			

(d) DOGS.

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding	Dogs :—		Intraperitoneal—*cont.*	Dogs :—	
	1	139		26	138
	3	139		114	139
	58	139			
	92	138			
			Subcutaneous	Dogs :—	
Intraperitoneal	Dogs :—			11	139
	15	140		74	138

(e) GOATS.

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding	Goats :—		Subcutaneous—*cont.*	Goats :—	
	20	123		3	125
	35	127		5	126
				7	127
				10	123
Subcutaneous	Goats :—			19	125
	1	126		28	124
	2	124		39	127

(f) RABBITS.

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding	Rabbit :—		Intraperitoneal—*cont.*	Rabbits :—	
	89	33		219	32
				231	32
				235	31
Intraperitoneal	Rabbits :—			238	32
	201	31		239	31
	212	32		240	32
	215	32		241	32
	217	32		250	31

(f) **RABBITS**—*cont.*

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Intraperitoneal—*cont.*	Rabbits :—		Intravenous—*cont.*	Rabbits :—	
	274	31		145	31
	278	31		152	31
	296	31		163	31
	298	31		535	31
	304	32			
	320	32			
	328	32	Subcutaneous.	Rabbits :—	
	346	32		83	31
				87	31
Intravenous	Rabbits :—			225	33
	Series (B III.)	25		563	33
	Series (H 7. C.M.)	25		564	31
	38	31		565	33
	72	31		567	33
	113	31		570	33

(g) **RATS.**

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding.	Rat :—		Intraperitoneal—*cont.*	Rats :—	
	20	152		39	150
				40	151
				42	144
Intraperitoneal	Rats :—			45	152
	13	148		47	144
	15	146		61	145
	24	150		73	145
	28	146			
	30	147			
	33	149	Subcutaneous.	Rats :—	
	34	148		14	149
	35	151		27	150
	36	148		B 37	144
	H 37	151		48	145
	38	151		55	145

(h) SWINE.

Method of Infection.	Designation of Animal.	Pages.	Method of Infection.	Designation of Animal.	Pages.
Feeding	Pigs :—		Feeding—*cont.*	Pigs :—	
	2	132		80	132
	8	129		92	133
	18	129		108	134
	20	129		114	134
	22	130			
	24	130	Subcutaneous	Pigs :—	
	26	130		21	135
	30	131		23	135
	34	131		94	133
	36	131		142	136
	38	131		144	135
	40	131		146	135
	42	132		148	135
	58	132		150	136

INDEX TO HISTOLOGICAL AND BACTERIOLOGICAL DETAILS.

VARIATIONS IN THE CULTURAL CHARACTERS OF INDIVIDUAL VIRUSES.

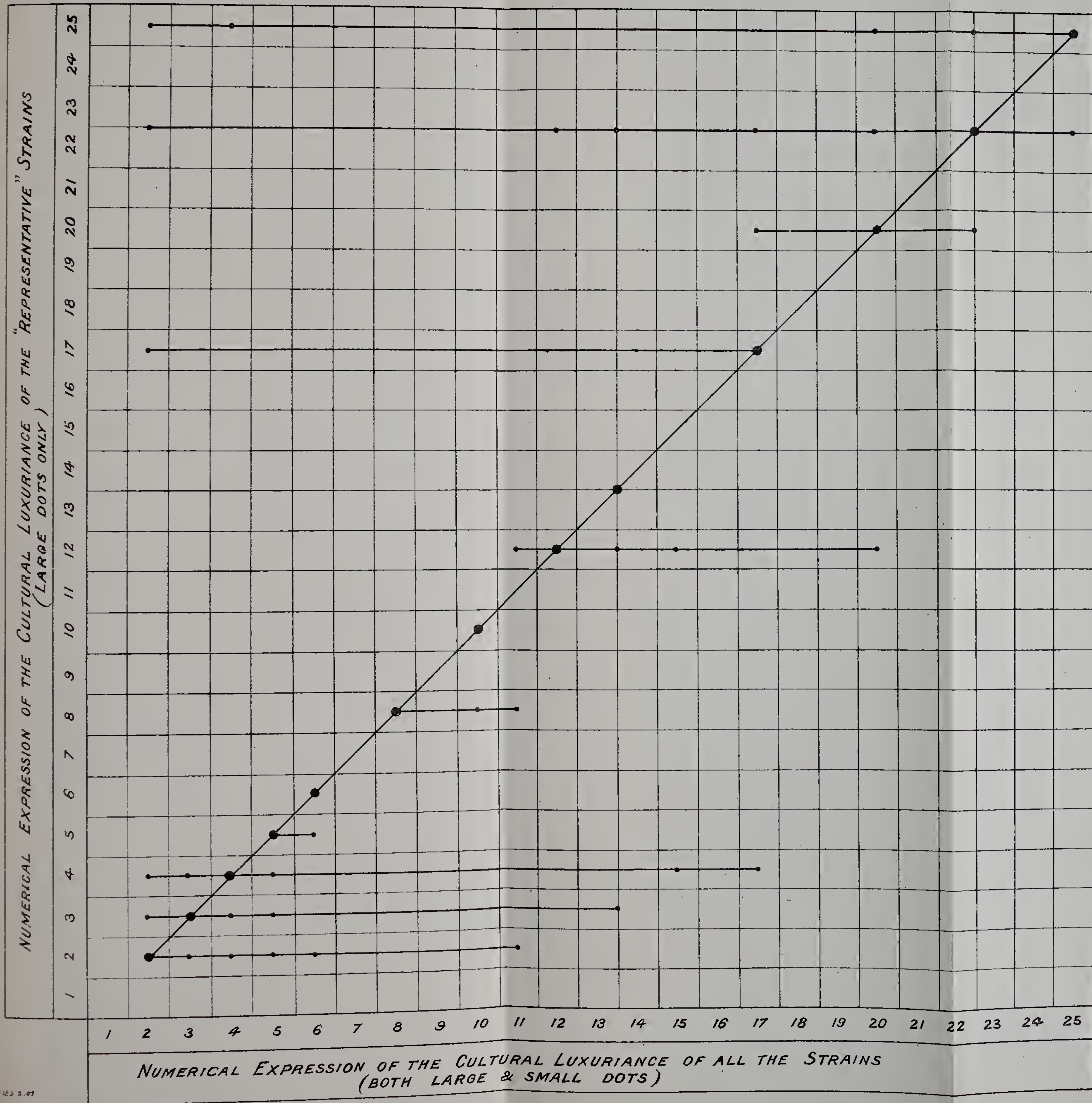

CHART I.

VARIATIONS IN THE CULTURAL CHARACTERS OF INDIVIDUAL VIRUSES.

The object of this Chart is to exhibit deviations (small dots placed on horizontal lines) from a standard (large dots joined by a diagonal line). The standard is determined as follows. Each large dot represents the standard of cultural luxuriance of all the viruses which are grouped together as possessing, according to their representative strains, identical degrees of cultural luxuriance. By a "representative" strain is meant a strain isolated from original material, when such was available, or, in default of such a strain, the strain nearest to the original material. On the diagonal line degrees of cultural luxuriance are measured both horizontally and vertically. Luxuriance increases, horizontally, as we pass from left to right; it increases, vertically, as we pass from below upwards. As the horizontal & vertical measurements are equal, each large dot may be regarded as forming the corner of a square. The numerical expression of cultural luxuriance is based on my culture classification, the increment of luxuriance exhibited by each of my five grades being expressed as 5 units. These values are, of course, diagrammatic, & no special emphasis is laid on precise numbers. Thus, all the cultures of Grades IV–V are taken as possessing one of four values, viz.— 17, 20, 22,–23, and 25. In Grades I–III a rather more minute subdivision has been found possible, the reason being that with the less abundant growths small differences are more readily appreciable than with the more abundant growths.

The small dots represent strains which diverge from the "representative" strains of the viruses to which they belong. They are placed, vertically, on the same level as their "representatives". Horizontally, their position is fixed by their amount of divergence from their "representatives", diminution of luxuriance being measured to the left and increase to the right.

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RELATION OF CULTURAL CHARACTERS TO VIRULENCE FOR CALVES.

SUBCUTANEOUS INOCULATIONS OF 50 MGS.

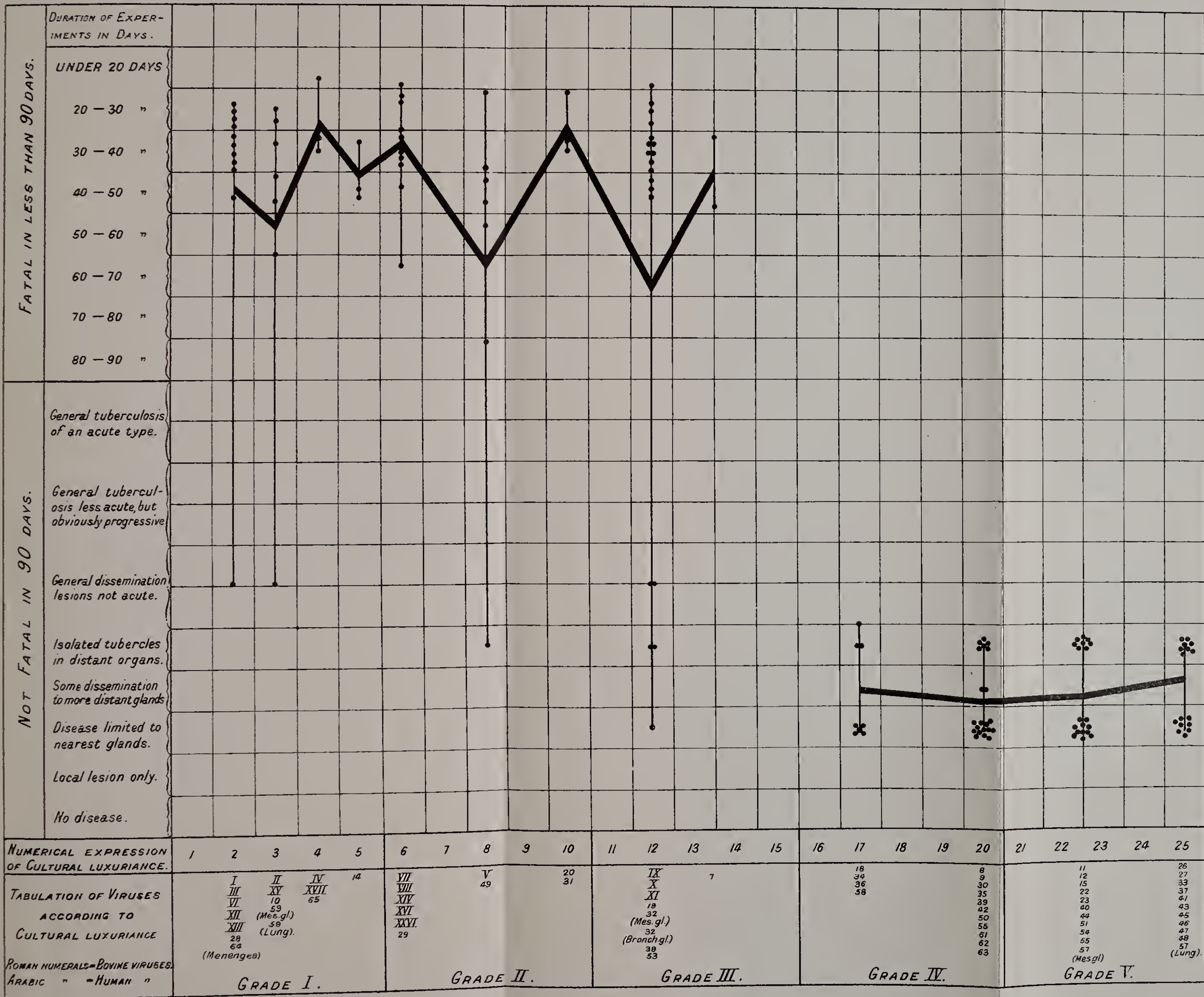

CHART II.

SUBCUTANEOUS INOCULATIONS OF 50 MGS.

This Chart represents the relation of cultural characters to virulence, the test of virulence being the effect of 50 mg. of culture subcutaneously inoculated into calves.

The cultural characters representative of each virus are those which were exhibited either by the strain isolated from original material, when available, or failing that, by the strain nearest to the original material. These strains are the same as those which are represented by large dots in Chart I.

Cultural luxuriance is measured horizontally, on the same scale and in the same way as in Chart I.

Virulence is measured vertically, the virulence increasing as we proceed from below, upwards. When the infection did not prove fatal within 90 days, the virulence is graded according to the amount and severity of the disease found post mortem. When the animals died (or were killed when very ill) in less than 90 days, virulence is graded according to the duration of the experiment.

Calves only are included (not older animals); cases in which infection was complicated by some other cause are omitted.

Each dot represents the result obtained on an experimental animal. The vertical position of the dot indicates the severity of the infection; the horizontal position indicates the cultural characters of the bacilli which caused the infection.

The vertical lines indicate the range of variation in virulence of viruses possessing any one standard of cultural luxuriance. At the foot of the columns in which these vertical lines occur, the viruses of which they are representative are tabulated.

From this chart four viruses are omitted, viz; H2. Sp. A., H13 A.D., H16 J.H., H17 Sp. B. The first of these is a case of mixed sputum from which no important conclusions can be drawn; the remaining three are exhibited in subsequent charts.

The thick transverse lines are obtained as follows— (1) Each vertical column is considered separately and the mean position or "centre of gravity" of the dots which it contains is ascertained. (2) By junction of the positions thus found the thick transverse lines are obtained.

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5189 66.

Relation of Cultural Characters to Virulence for Calves.

Subcutaneous Inoculations of 10 mgs.

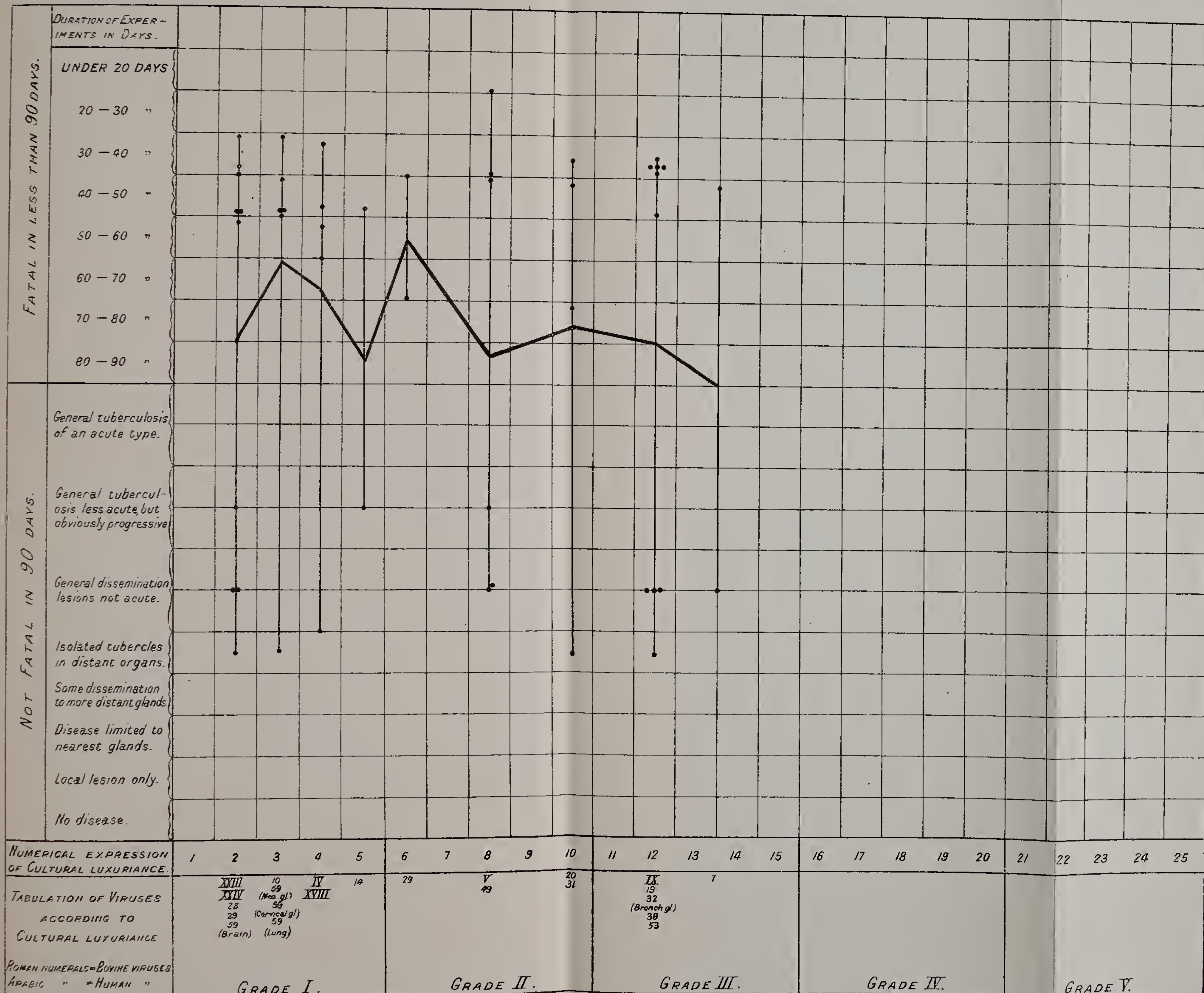

Chart III.

Subcutaneous Inoculations of 10 mgs.

This chart has been drawn up in exactly the same way as chart II, the only difference being that the test dose for virulence is 10mg of culture instead of 50 mg.

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Relation of Cultural Characters to Virulence for Calves.

Subcutaneous Inoculations of Tissue Emulsions.

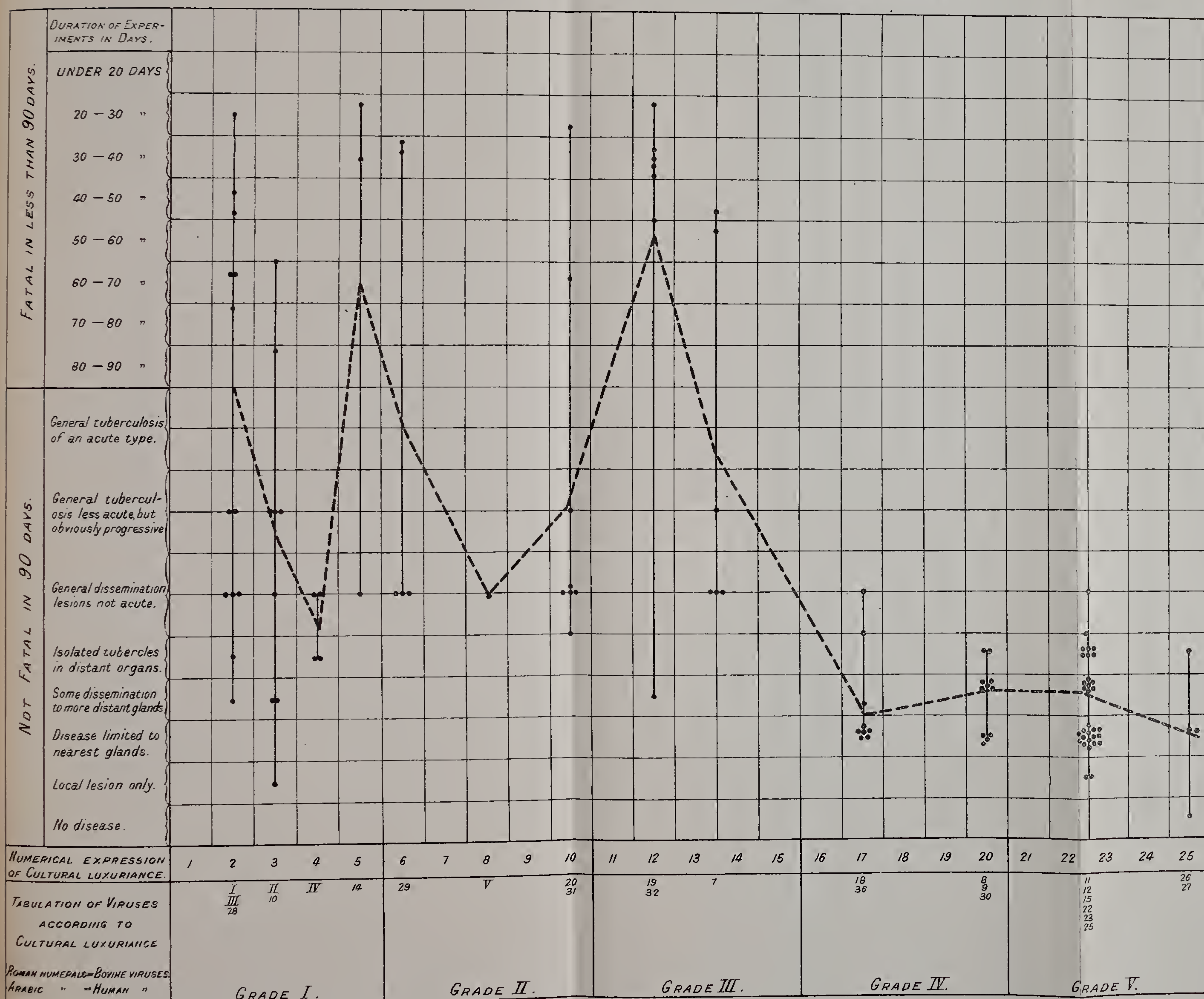

Chart IV.

Subcutaneous Inoculations of Tissue Emulsions.

This chart is drawn up in the same way as Charts II and III, the difference being that the test doses for virulence consisted of bacilli contained in emulsions of animal tissues.

In most of these experiments the number of bacilli contained in the emulsions has been estimated, but no uniformity of dosage has been adopted. This fact must be carefully borne in mind; otherwise, the chart might be erroneously interpreted.

It will be observed that all the columns which rise to a high degree of virulence, (infection fatal in less than 90 days) are in the left half of the chart. Confining our attention to these columns, the following facts are noteworthy. Severe infection (fatal in less than 90 days) has been produced (1) by small doses (e.g. doses estimated at about 50 thousand, 100 thousand, and 250 thousand bacilli), (2). by medium doses. (e.g. doses estimated at about 1½, 2½, and 4½ million bacilli), and (3) by large doses (e.g. doses estimated at about 240, 650, and 900 million bacilli). On the other hand, relatively mild infection (i.e. not graded above "General dissemination; lesions not acute.") is also charted in these same columns as the result of doses which have sometimes been (1) small (e.g. 9 thousand, 116 thousand and 200 thousand bacilli), (2) medium (e.g. 1, 2, and 4 million bacilli) (3) large (e.g. 10, 11, and 355 million bacilli.).

In the right half of the Chart the columns never rise above the level — "General dissemination; lesions not acute." Confining our attention to these columns, the following facts are noteworthy. In 6 Cases the dose was under 1 million bacilli; in 19 Cases it was between 1 and 10 millions; in 14 Cases it was between 10 and 100 millions and in 19 Cases it was over 100 millions.

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Relation of Cultural Characters to Virulence for Calves.

Modifications observed in the Virus H 13. A.D.

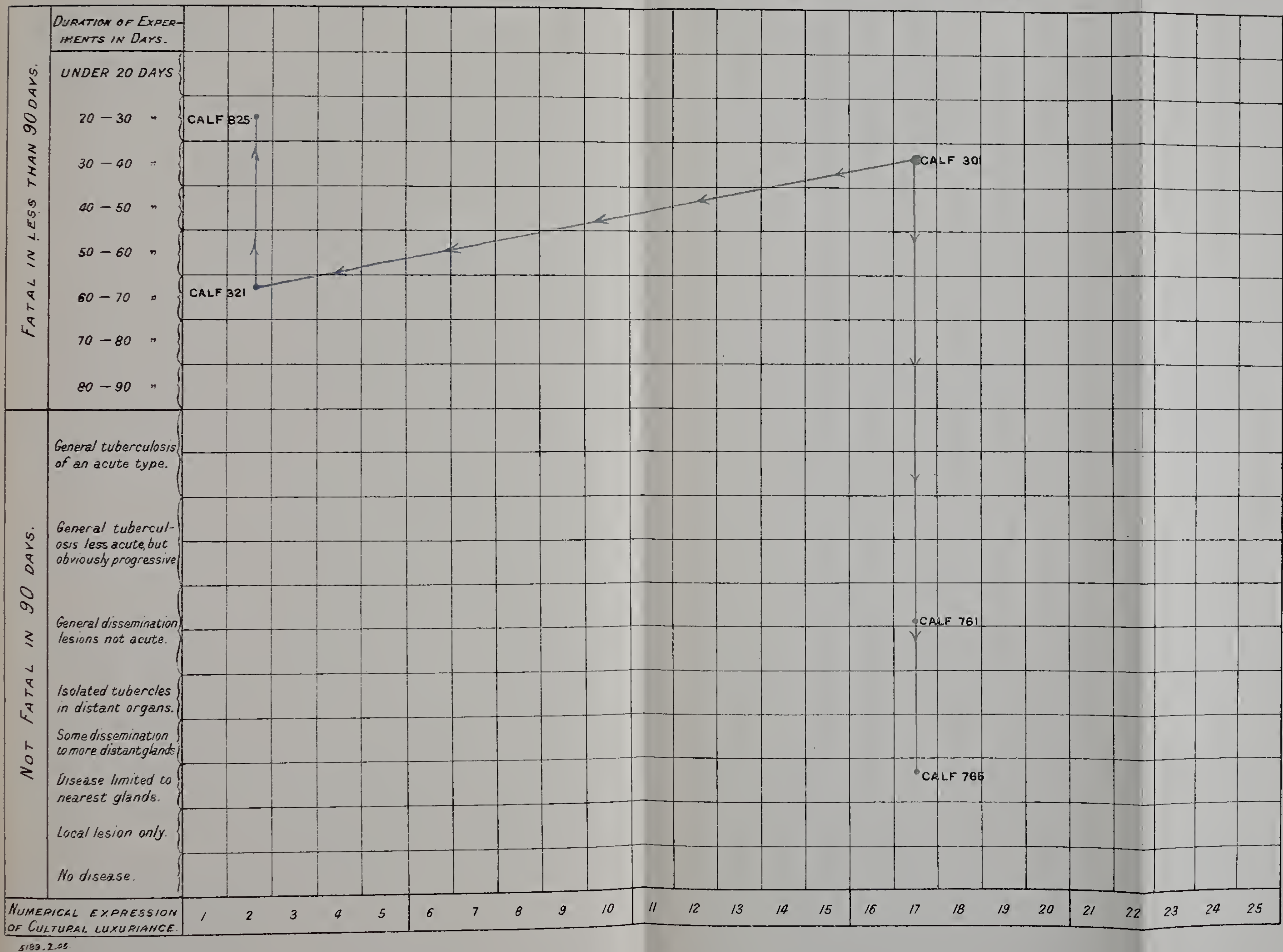

Chart V.

Modifications Observed in the Virus H 13. A.D.

The modifications observed in this virus commence with Calf 301.

The position assigned to Calf 301 in the chart indicates that it was fatally infected in 33 days and that the bacilli recovered from it exhibited a high degree of cultural luxuriance.

The line passing vertically downwards from Calf 301 indicates that the cultures obtained from this animal were, 15 months after isolation, inoculated into Calves 761 and 765, in doses of 50mg.

The vertical positions assigned to Calves 761 and 765 indicate the slight amount of disease produced in these two animals.

The line passing from Calf 301 transversely, from right to left, indicates that bacilli contained in the tissues of Calf 301 were transferred to Calf 321.

The position assigned to Calf 321 indicates that it was fatally infected in 63 days and that the bacilli recovered from it exhibited a very low degree of cultural luxuriance

The line passing vertically upwards from Calf 321 indicates that 50mg of a culture from this animal was inoculated, 15 months after isolation, into Calf 825.

The position assigned to Calf 825 indicates that this animal was fatally infected in 25 days.

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Relation of Cultural Characters to Virulence for Calves.

Modifications observed in the Virus H 16. J. H.

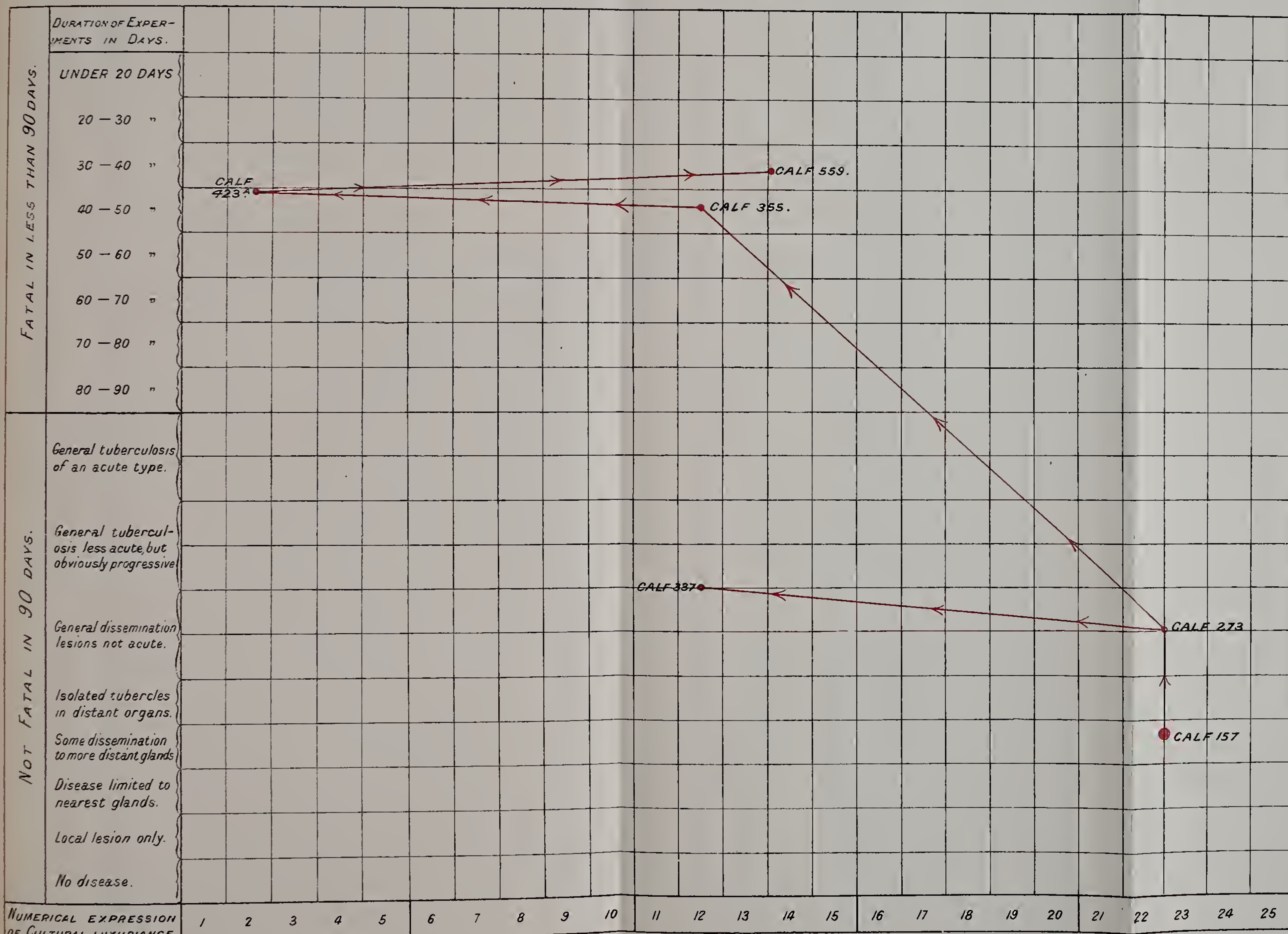

Chart VI
Modifications Observed in the Virus H 16. J. H.

The process of modification observed in this Virus commences with Calf 157.
The position assigned to Calf 157 indicates that it was only slightly infected and that the bacilli recovered from it exhibited a high degree of cultural luxuriance.
The line passing vertically upwards from Calf 157 indicates that bacilli contained in the tissues of this animal were passed through Calf 187 and thence into Calf 273.
The position assigned to Calf 273 indicates that this animal was not severely infected & that the cultures obtained from it were as luxuriant as those of Calf 157.
The line passing transversely to the left from Calf 273, indicates that bacilli from the tissues of this animal were passed into Calf 337.
The position of Calf 337 indicates that this animal was infected with moderate severity & that the cultures obtained from it grew with much less luxuriance than those of Calf 273.
The line passing upwards & to the left from Calf 273, indicates that bacilli from the tissues of this animal were passed into Calf 355.
The position of Calf 355 indicates that this animal was fatally infected in 43 days & that the bacilli recovered from it exhibited cultural characters identical with those of Calf 337.
The line passing tranversely to the left from Calf 355, indicates that bacilli from the tissues of this animal were passed first into Rabbits & thence into Calf 423A.
The position of Calf 423A indicates that this animal was fatally infected in 42 days & that the bacilli recovered from it exhibited a very low degree of cultural luxuriance.
The line passing transversely to the right from Calf 423A indicates that 50 mg. of a culture from this animal were inoculated into Calf 559.
The position of Calf 559. indicates that this animal was fatally infected in 39 days & that the cultures obtained from it grew with a degree of luxuriance intermediate between Calf 423A and Calf 273.

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Modifications observed in the Virus H. 17. Sp. B.

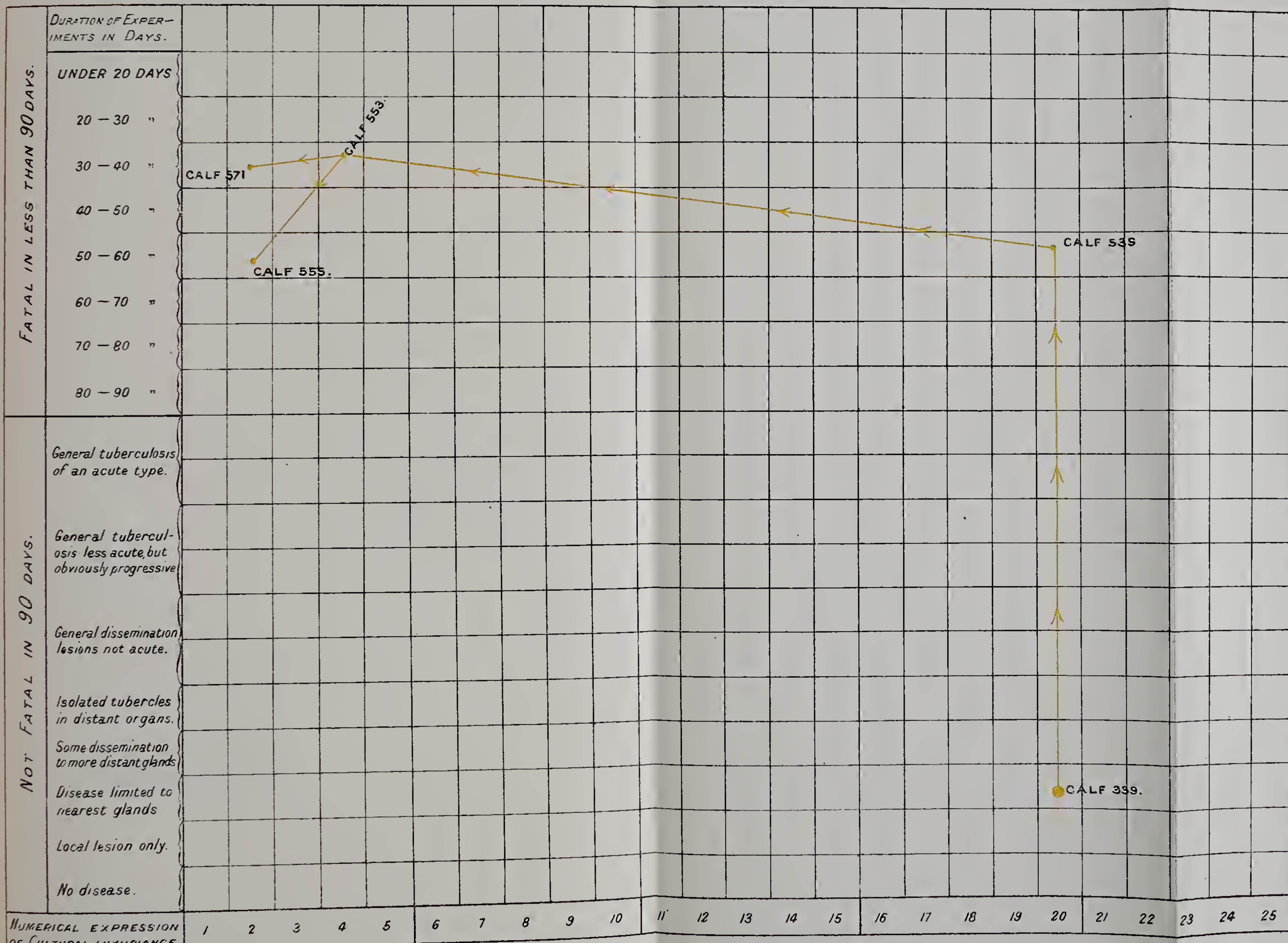

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Chart VII
Modifications Observed in the Virus H. 17. Sp. B.

The modifications observed in this Virus commence with Calf 339.
The position of Calf 339 indicates that this animal was only slightly infected & that the Cultures obtained from it possessed a high degree of luxuriance.

The line passing vertically upwards from Calf 339 indicates that bacilli from the tissues of this animal were passed first into Guinea-pigs, then, intravenously, into Calf 475, then, intravenously, into Calf 539.

The position of Calf 539 indicates that Cultures from this animal, obtained through Rabbit 181, produced fatal infection in Calves (in subcutaneous doses of 50 mg.) in 48 and 58 days (Average 53 days) & that these Cultures exhibited the same degree of luxuriance as the Cultures of Calf 339.

The line passing transversely to the left from Calf 539 indicates that bacilli from the tissues of this animal were passed, intravenously, into Calf 529 & thence, intravenously, into Calf 553.

The position of Calf 553 indicates that Cultures obtained from this animal produced fatal infection of a Calf (in a subcutaneous dose of 50 mg.) in 32 days, and that these Cultures possessed a markedly low degree of luxuriance.

The line passing downwards & to the left from Calf 553 indicates that bacilli from the tissues of this animal were inoculated subcutaneously into Calf 555.

The position of Calf 555 indicates that this animal was fatally infected in 58 days & that bacilli isolated from it possessed a very low Cultural luxuriance.

The line passing transversely & to the left from Calf 553 indicates that bacilli from the tissues of this animal were passed first, intravenously, into Calf 557, and thence, intravenously, into Calf 571.

The position of Calf 571 indicates that Cultures isolated from this animal produced fatal infection in Calves (in subcutaneous doses of 50 mg) in 33 and 37 days (Average 35 days), and that these Cultures grew with a very low degree of luxuriance.

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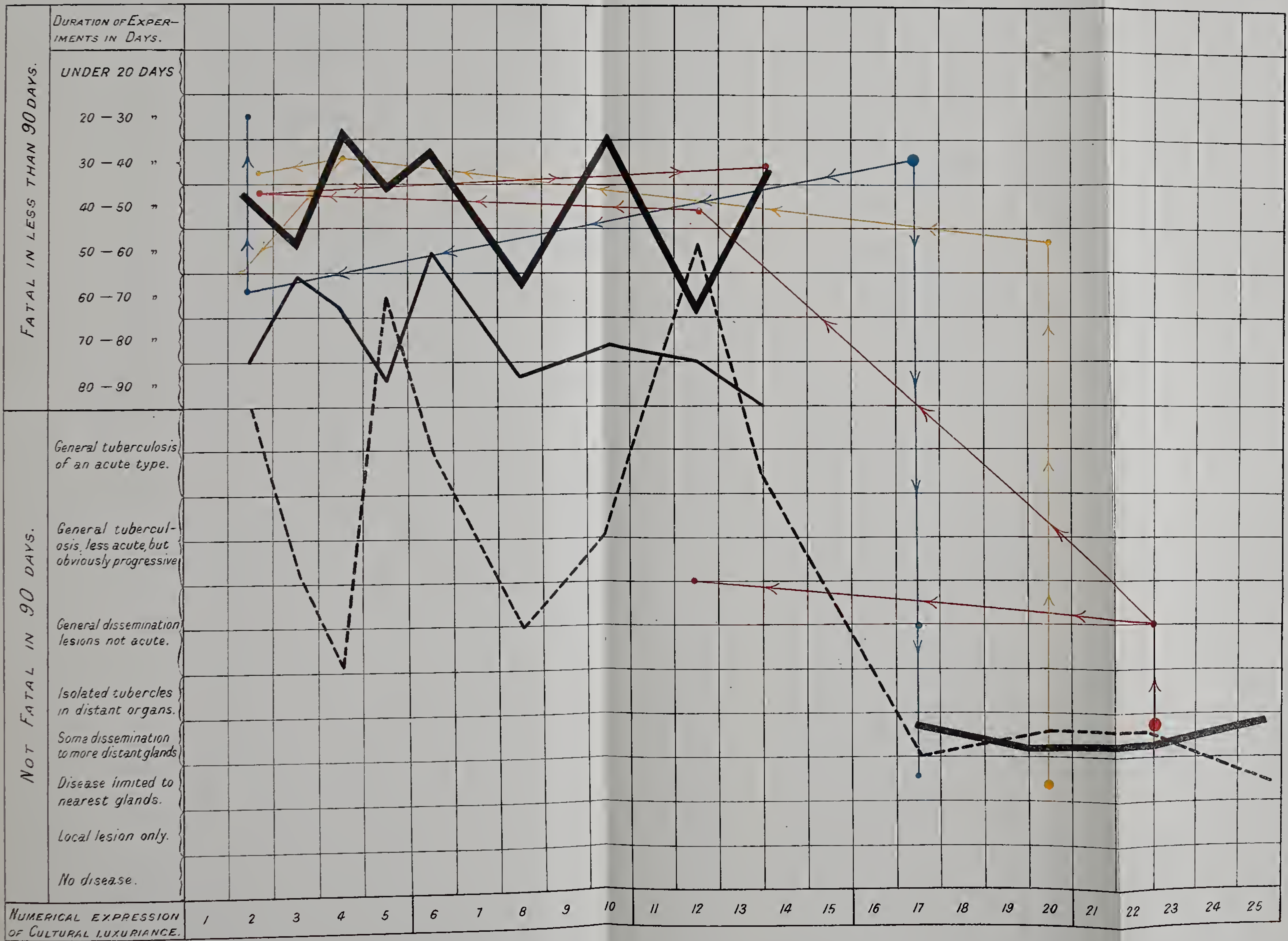
The Combined Results of Charts II–VII.



Charts VIII.
The Combined Results of Charts II–VII.

In this chart the results of the six preceding charts are superimposed upon one another.

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